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FINAL REPORT

EVALUATION OF LIQUID STERILANTS

Submitted in fulfillment
of
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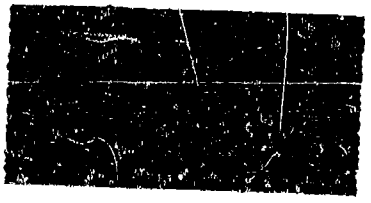

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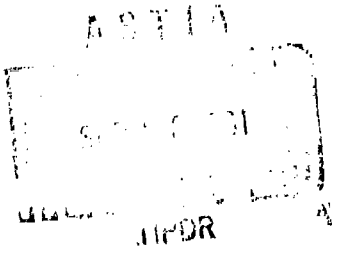

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I. INTRODUCTION

That sterilization of spacecraft to avoid contamination of other heavenly bodies with terrestrial microorganisms is an important and necessary part of space exploration has been generally accepted. The actual attainment of sterility in constructing and launching spacecraft, however, presents many problems not usually associated with medical and pharmaceutical sterilizing procedures in general use today. Because assembly of such a large, complicated piece of apparatus as a spacecraft from sterilized components in an absolutely sterile technique would be impractical, some compromise between assembly by sterile technique and terminal sterilization of the final assembly is needed. While terminal sterilization by dry heat at 125°C for 24 hours appears to offer the most straightforward and least expensive solution to the problem, it cannot be put into practice at present because many of the individual components of several of the proposed spacecraft will not function reliably if treated in such a manner.

The procedure being considered for use in sterilizing spacecraft to be launched in the near future consists of the fabrication of sterile subassemblies, mounting them on the spacecraft framework, and sterilizing the exposed external surfaces of the final assembly with gaseous ethylene oxide. For such a technique to be successful, however, the occluded surfaces between the subassemblies and in the framework and any other surfaces which cannot be reached by gaseous ethylene oxide must be sterilized during the final assembly process. The use of liquid sterilants on these surfaces would be the ideal solution if effective procedures for using such liquids could be developed which do not present corrosive or destructive possibilities.

This report presents the results of a study to evaluate several chemicals which might serve as liquid sterilants. This study included evaluation of the compatibility of the chemicals with a wide variety of materials as well as an evaluation of their sterilizing effectivenesses.

The selection of the chemicals investigated was based on the work of C. R. Phillips[1] with ethylene oxide and related compounds. The five chemicals selected were used with six common solvents as vehicles. The solvents were selected to represent a wide range of physical properties of solvents. The purpose of the study was to evaluate the chemicals from both the sterilizing and compatibility standpoints with respect to a wide variety of materials. The experimental plan to produce the desired information without a prohibitively large number of tests was designed in accordance with modern statistical interpretation procedures in mind. The program was divided into two phases.

In Phase I, the relative effectivenesses of the thirty chemical-vehicle combinations in sterilizing a magnesium alloy strip with a Dow 7 surface treatment was determined while holding all other factors fixed at arbitrary levels. Phase I was designed to reduce to manageable proportions the total number of combinations to be studied further. Implicit in the design of Phase I is the assumption that the effect of all factors other than chemical and vehicle are negligible in comparison with the effects of these factors. The evaluation of the effects of temperature of application, of ratio of amount of chemical to vehicle, and of duration of exposure has been beyond the scope of the present work. The results reported here apply only to one microorganism, B. subtilis, var. niger, and then only to one particular strain of this organism.

Because of the high volatility of most of the vehicles studied and the low volatility of most of the chemicals, the relative concentrations of these materials in the liquid phase changes continuously during evaporation. This change may be so important in either or both the achievement of sterility or the attainment of reliable performance that rate and extent of evaporation will have to be carefully controlled when such liquid sterilants are used in manufacturing operations.

In Phase II, the effectiveness and compatibility of four candidate sterilants

5% v/v beta-propiolactone in distilled water

5% v/v beta-propiolactone in Solvent M-17 (J. B. Moore Co. product)

5% v/v ethylene imine in trichloroethylene

5% w/v formaldehyde in methanol

were evaluated with respect to a number of objects representing a variety of surfaces, materials, and configurations. The sterility tests were directed toward measuring the extent to which known populations of B. subtilis, var. niger spores were reduced in size, rather than to demonstrating total absence of viable microorganisms. From the results of these tests, absolute sterility tests can be designed with considerably more confidence than could be done without such information.

The results of this study confirm the feasibility of developing a satisfactory and reliable process, using a liquid sterilant comprised of one of the chemicals studied in one of the vehicles studied, for sterilizing a specified surface. They are, however, insufficient to demonstrate that

any particular application of such a liquid sterilant to any unspecified surface will make that surface sterile or will not damage the material or component under that surface. It is certainly true, however, that any reasonable process designed soley from the information presented in this report will reduce the viable microorganism population on any surface substantially. On most thoroughly cleaned surfaces, handled aseptically, application of such a process will sterilize the surface. If one is interested in identifying specifically those surfaces which will not be sterilized, and certainly some of them will not be by application of such a process, the presently available information is insufficient to identify those surfaces.

Studies of toxicity, explosion hazards, and handling problems of the liquid sterilants were beyond the scope of this study. It should be emphasized that such studies should precede the adoption of these liquid sterilants for routine use.

II. PHASE I

The purpose of Phase I of this evaluation was to reduce the number of candidate sterilants from thirty to four in order that these four could be evaluated more thoroughly in Phase II. The relative effectivenesses of the thirty chemical-vehicle combinations, chosen initially as candidate sterilants, may be inferred from the information presented in Table I and in subsequent tables which have been derived from Table I. The chemical stability and the volatility of these same mixtures may be inferred from Tables VIII through XI of Phase I.

A. METHODS

Except for formaldehyde, the five chemicals, specified below, were dispersed in each of the six vehicles in a concentration of five milliliters of chemical in each 100 milliliters of chemical-vehicle mixture, at room temperature (this concentration corresponds to 5% v/v, in accordance with the definitions in USP XVI)[2]. Formaldehyde 37%, USP, was used in the amount of 13.51 milliliters in each 100 milliliters of chemical-vehicle mixture. This amount gave 5% w/v of formaldehyde in the mixture. The results in Table I suggest that the methanol in the formaldehyde 37%, USP, contributed to the sterilizing effect of the formaldehyde containing liquids. Formaldehyde 37%, USP, contains about 10% methanol. The remainder of the liquid is water.

CHEMICALS

1. Ethylene imine, Matheson Coleman & Bell, #Ex 580, Lot 7291
2. Epichlorohydrin, Matheson Coleman & Bell, #Ex 55, ST 2637
3. Epibromohydrin, Eastman #3429, No Lot Number
4. Beta-propiolactone, Eastman #6662, No Lot Number
5. Formaldehyde, 37%, U.S.P., Braun Chemical Co., Lot Number 003

VEHICLES

a. 20 grams of Tide (household detergent) per liter dissolved in distilled water (water for injection, U.S.P.)

b. Trichloroethylene, "Baker Analyzed" Reagent #9458, Lot No. 23110

c. Acetone, 99.5% purity, "Baker Analyzed" Reagent #9006, Lot No. 23574

d. Methanol, Absolute, "Baker Analyzed" Reagent #9006, Lot No. 23270

e. Solvent M-17, John B. Moore Co., furfasol, S. O. #LA 8400 E (contains tetrahydrofuran, dichloromethane, and trichloroethylene)

f. Solvent M-50, John B. Moore Co., 1, 1, 1-trichloroethane, S. O. #LA 8400 E (contains 1, 1, 1-trichloroethane and trichloroethylene)

The chemical-vehicle combinations were used to cover inoculums of one million viable spores of Bacillus subtilis, var. niger residing on one surface of a magnesium alloy strip. The spores were suspended in distilled water and while so suspended they were deposited, as a single drop on the strip. The volume of the inoculum was 0.01 milliliters and was metered by

a tuberculin syringe. While at room temperature, the water was permitted to evaporate from the spores and the strip into the surrounding air. During this drying operation, the inoculated strips were lying in a clean, covered, sterile, glass Petri dish with the inoculated side up. The Petri dish also contained the replicate strip and a bacteriostasis control strip, which was not inoculated.

The magnesium alloy strips were 1-1/4 inches wide by 3 inches long by 0.016 inches thick. They were prepared of alloy AZ31B and have a machined surface finish, 125 microinches rms or better, and a Dow 7 surface treatment. They were identical with the specimens of subject a of Phase II. Before inoculation, each strip was dipped in clean acetone, reagent, in the tank of a small ultrasonic cleaner. The strip was permitted to dry completely in the air before being placed, with its replicate and bacteriostasis control strip in a clean, covered, glass Petri dish. The strips were sterilized in the Petri dishes in a dry air oven at 125°C for 24 hours. The Petri dishes and the three strips inside each of them for each of the following chemical-vehicle combinations were autoclaved in saturated steam at 120°C for one hour before being exposed to dry air at 125°C for 24 hours.

Ethylene imine in 2% w/v Tide in water

Ethylene imine in Solvent M-50

Epichlorohydrin in Acetone

Epichlorohydrin in Trichloroethylene

Epibromohydrin in Trichloroethylene

Formaldehyde in Solvent M-17

Beta-propiolactone in Solvent M-17

The dry spores of B. subtilis, var. niger which were supplied by Jet Propulsion Laboratory were originally obtained from the Microbiological group at Ft. Detrick. They were suspended in distilled water at a concentration of one billion per milliliter. This suspension constitutes the stock suspension discussed subsequently in this report. A 10 to 1 dilution of the stock suspension was prepared for use in inoculating specimens for the sterility tests of both Phases I and II. In this suspension, the concentration of spores which demonstrated viability when incubated on Trypticase soy agar was measured and found to be consistent with the designed concentration of one hundred million per milliliter. The volume of the suspension required to deposit an inoculum of one million demonstrably viable spores was calculated to be 0.01 milliliter. The assay of viable spore concentrations are reported in Table V.

After washing, all spore handling equipment was sterilized in saturated steam at 120°C for one hour before use or re-use.

When first received, the five chemicals and the six vehicles were subjected to identity tests such as odor and infrared spectra. The combinations were prepared as one uniform batch which was used throughout the testing in Phase I.

In turn, each of the thirty chemical-vehicle combinations was sprayed on the inoculated surfaces of three magnesium alloy strips in each Petri dish while these strips were lying horizontally. The spray was applied with a polyethylene atomizer* on the inoculated sides of the strips only and on just

*Royal, #24-410 Modified Cylinder Rounds in linear polyethylene; Royal, #5-1950 White Polyethylene side-spray hinge caps; Royal, Natural Polyethylene spray tubing.

the top side of the bacteriostasis control strip. A different atomizer was used with each chemical-vehicle combination. The chemical-vehicle combinations were stored in the polyethylene atomizers. This operation produced two specimens of each of thirty chemical-vehicle combinations applied to an inoculated magnesium alloy strip, and one specimen of each on an uninoculated strip. Two additional inoculated alloy strips, called "Control 2", were set aside without being exposed in any way to any possibly sterilizing environment.

After being sprayed, each strip remained continuously for 90 minutes inside its Petri dish in a horizontal position. The gas in the Petri dish was saturated with the chemical-vehicle combination though most of the liquids evaporated long before the 90 minutes had expired.

At the end of 90 minutes, each magnesium alloy strip in its turn, including Control 2 strips, was transferred, aseptically, to a sterile glass jar, containing 20 milliliters of sterile distilled water and closed with a sterile closure. During the transfer, this strip was handled by sterile forceps only. The tightly closed jar containing the strip and water was immersed for 1 minute in water subjected to ultrasonics at 40 kc and 50 watts.

Within a few minutes after being removed from the ultrasonic field, 1.4 milliliters of the liquid in the jars containing the inoculated strips were withdrawn into a clean sterile 5cc glass syringe. A 0.2 milliliter aliquot was expelled from the syringe onto the surface of a sterile Trypticase soy agar plate. A second 0.2 milliliter aliquot was expelled from the syringe onto the surface of a second sterile Trypticase soy agar

plate and the remaining one milliliter aliquot was expelled into nine milliliters of sterile distilled water inside a sealed vial which had been sterilized with the water in it.

Both the jars containing the 20 milliliters of distilled water and the vials containing the 9 milliliters of distilled water were prepared by Horton and Converse, Los Angeles, in order to obtain maximum assurance of sterility and purity of the water.

Five milliliters of the contents of the vial were withdrawn into the syringe and then returned to the vial. Four additional withdrawals and returns were made. While the dilution factor is not precisely 10 when this technique is used, the results are reproducible and the chances for mistakes are few. One and four tenths milliliters of the contents of the vial were then drawn into the syringe. A 0.2 milliliter aliquot was expelled onto the surface of a third sterile trypticase soy agar plate. A second 0.2 milliliter aliquot was expelled onto the surface of a fourth sterile Trypticase soy agar plate and the remaining one milliliter aliquot was expelled into nine milliliters of sterile distilled water inside a second closed sterile vial. The process of mixing, withdrawing 1.4 milliliters, expelling onto the sterile Trypticase soy agar plates and into nine milliliters of sterile distilled water in a third vial was repeated in a manner identical to that done with the first vial.

The same steps were repeated for the third vial with the exception that only 0.4 milliliters were withdrawn because only the two sterile Trypticase soy agar plates, numbers 7 and 8, were to receive 0.2 milliliter of liquid each. By nutation, the liquid on the surfaces of all the Trypticase soy agar plates was distributed as uniformly as possible.

Each specimen was processed in this identical manner to produce eight test plates, except for the bacteriostasis controls where plates 1, 2, 3, and 4, were not prepared because bacteriostasis can be expected here in several cases. To those vials prepared using strips which had never been inoculated, the bacteriostasis controls, 0.01 milliliters of the spore suspension containing one hundred million spores per milliliter were added. The contents of the inoculated vials were then plated, 0.2 milliliter to each of two plates for each dilution. The expected number of colonies on each plate was 10^4.

The Trypticase soy agar plates for Control 1 were prepared by adding the standard inoculum, as applied to each of the magnesium alloy strips, directly to a jar containing twenty milliliters of sterile distilled water without having ever been exposed to either the magnesium alloy strip or a chemical-vehicle combination. The jar was then tightly closed and shaken vigorously. The liquid in the jar was then "plated" as though a magnesium strip had been placed in the jar. The numbers of colonies which developed on the plates are reported in Table V. Two different jars were inoculated and plated at the start of Phase I. Additional assays were made during Phase II.

The jar which received the inoculum was called "1". After one milliliter of its contents was transferred to a vial for serial dilution (call these vials "set 1") this jar was placed in water in an ultrasonic field for one minute and then a second set of serial dilution vials and plates were prepared from its contents. The numbers of colonies which

developed on the plates are reported in Table VI opposite the entry "ultrasonics." Comparison of the Control 1 entries with those opposite ultrasonics indicates that exposure to ultrasonics had no substantial effects on the viability of the spores.

The vials of "set 1" were exposed to 60°C for 10 minutes and then plates were prepared from them. The numbers of colonies which developed on the plates are reported in Table VI opposite the entry "heat shock."

The plates were incubated at 37 ± 2°C for from three to seven days. The number of colonies which developed on each of the plates were counted and recorded in Table I. The colonies were verified to be B. subtilis, var. niger by colony and cell morphology and Gram-staining as was appropriate.

The colonies of B. subtilis, var. niger were counted on the day following the preparation of the plates. Counting on the third day did not change the recorded results. The colonies are at the most convenient size to count 24 hours after the plate is prepared. After three days, colonies grow together to a large extent. Of course, plates with no or few colonies must be verified to contain no colonies again after seven days incubation before confidence may be placed in these zero results.

Comparison of the colony count of Control 1 plates with those representing exposure to ultrasonics indicates that the exposure to ultrasonics had no substantial effect on the viability of the spore inoculum. Comparison of Control 1 and Control 2 colony counts discloses no sterilizing effect of the magnesium alloy or its surface treatment independently of

exposure to the "sterilizing treatment" and demonstrates the effectiveness of the rinsing method, using ultrasonics and twenty milliliters of distilled water, for removing viable spores from the surface of the magnesium alloy strip.

B. STERILITY TEST RESULTS AND DISCUSSION

1. ANALYSIS OF VARIANCE

The inevitable presence of variation has been long recognized as a characteristic difference between the biological and the physical sciences. In biological sciences, the comparison of the variation in effect produced by controlled variables (factors) with the variation produced by random uncontrolled factors represents the principal component in quantitative measurements. Analysis of variance[3] is a formal, and logical, procedure for making this comparison. In an analysis of variance, the sum of the squares of the deviations of the measurements from the mean of all the measurements is divided into several independent estimates of the variance (a precise mathematical concept as used here) which may be assigned to the measurements if the controlled variables had no effects on the measured quantity. Each of these independent estimates of the variance is assigned to a controlled variable (factor) by a formal procedure. These estimates of the variance are then compared with the residual estimate (the total variance estimate less the sum of the individual variance estimates assigned to controlled factors) by application of the F-test. If the variance

assignable to one of the factors is significantly larger than the residual estimate of the variance, then the factor is said to be significant because the variation in the measurements assignable to the factor was larger than could be reasonably accounted for by the effects of random uncontrolled factors.

In the Analysis of Variance tables presented in this report, one takes the ratio of the mean squares (MS) as the ratio F. From the relative magnitudes of the mean squares, the relative importances of the several factors in the total variation among the data may be inferred.

The data obtained in Phase I was subjected to an analysis of variance. This analysis permits an inference to be made about the significance of the results. The analysis of variance indicates that the differences in sterilizing effectiveness among the several conditions were highly significant for change from one chemical to another, but were not significant with respect to change from one vehicle to another. The variation which was due to differences in the numbers of viable spores recovered* from replicate strips was as large as the variation arising from change from one vehicle to another. While it may be that the vehicles do have different effects, the precision of the measuring technique was not sufficient to detect them. The interactions between chemicals and vehicles have a greater effect on the sterility test results than do the vehicles themselves.

*Differences may be due to variation in effective exposure as well as to thoroughness of the process of rinsing the spores off the strips.

The measure of sterilizing effectiveness was assumed to be the sum of the numbers of colonies developed on the plates for the $1:10^1$, $1:10^2$, and $1:10^3$ dilutions. Table II presents the numerical values of these sums. This measure is not unique and may not even be the best. It is useful, however, in that it ranks the various combinations of chemicals and vehicles in inverse order of their sterilizing effectivenesses. The analyses of variance presented in Tables III and IV permit one to compare the variation of results arising from variation in the controlled factors, with the variation in the results arising from the experimental techniques.

In Table I, there is a footnote pointing out that the colony counts for the second replicate for 5% v/v epichlorohydrin in Solvent M-50 are, possibly, unreasonably low. Table III has been calculated on the basis that the information presented in Table I does represent reality and Table IV is calculated on the basis that the data for the second replicate are identical with those of the first replicate. This adjustment of the data has a pronounced effect on the distribution of the variance between the plates and the strips and results in a possibly more accurate picture of the experimental technique. This adjustment of data, however, does not affect the general conclusions to be drawn from the analysis of the variance, which is, that for purposes of sterilizing B. subtilis, var. niger spores, the differences among the effects of the chemicals are

much greater than are the differences among the effects of the vehicles or the interactions of the chemicals with the vehicles. The autoclaving of certain of the magnesium alloy strips prior to inoculation did not appear to affect the results of the sterility tests.

2. SPORE SUSPENSION ASSAYS

A 10 to 1 dilution of the stock suspension was prepared for use in inoculating specimens for the sterility tests in Phase I. In Table V, the results of the assays on this 10 to 1 dilution are reported as the 6-8 and 6-9 assays. These assays, which are also called "Control 1" in the subsequent discussion, are consistent with the presumption that the stock suspension contains 10^9 spores of B. subtilis, var. niger per milliliter. The volume of the 10 to 1 dilution of the stock suspension required to deposit an inoculum of one million demonstrably viable spores is then 0.01 milliliters. Other assays of the viable spore content of the stock suspension are reported in Table V. The spores appear to maintain their viability while suspended in distilled water for a period of two months under storage at $7^{\circ}C$.

3. VIABLE SPORE RECOVERY CONTROL

The number of viable spores recovered from the magnesium alloy strips not exposed to candidate sterilants are shown in Table V and in Table I of Phase II as the entries for Control 2 of subject a and in Table VIII of Phase II. (In Phase II, the magnesium alloy strip is designated as subject a.) The rinsing operation recovered from the magnesium alloy strips nearly all of the inoculum in the Phase I tests. In the Phase II tests, however, the recovery was between 8 and 10% of the viable spore inoculum.

4. BACTERIOSTASIS CONTROL

In Table I, the bacteriostasis control plates in every instance indicated that insufficient amounts of the chemical remained in the higher dilutions to prevent proliferation of large inoculums of spores known to be viable.

5. HEAT SHOCK AND ULTRASONICS

In an effort to obtain maximum recovery of viable spores from magnesium alloy strips, the inoculum was scrubbed and rinsed into sterile distilled water by ultrasonic scrubbing of the strip. Table VI compares the Control 1 assay, same as reported in Table V, with similar assays of the same spore suspension after it had been immersed

in an ultrasonic bath, for one minute, in a manner identical with that used in scrubbing the magnesium alloy strips. The ultrasonic irradiation at 40 kc and 50 watts, of itself, does not appear to affect the viability of the spores. A separate portion of the spore suspension used in the Control 1 assays and the ultrasonic assays was heat shocked in an effort to improve, if possible, the proportion of the spores which would germinate when placed on nutrient agar and incubated. The data in Table VI failed to indicate that any increase in the proportion germinating was attained by heat shock.

6. RESISTANCE OF SPORES

The particular strain of B. subtilis, var. niger used in this work was selected because of the existence of a large amount of experimental information obtained on its resistance to exposure to ethylene oxide by the Microbiological group at Ft. Detrick. This strain represents, therefore, a resistant microorganism of known characteristics. One of the authors has had considerable experience with the behavior of a different strain, BS4 of American Type Culture Collection No. 9372, in gas sterilization experiments. Table VII presents the results obtained when identical inoculums of these two organisms were exposed to the ethylene oxide process, used in Phase II, for three hours. The BS4 strain may possibly be

more resistant to ethylene oxide, on the basis of the information shown in Table VII, and thereby more resistant to the several candidate sterilants considered in the present work. Because the two spore inoculums were handled identically, differences in the state of hydration probably were not significant.

C. CHEMICAL STABILITY, RESULTS AND DISCUSSION

Because the chemicals used in this evaluation are known to be highly reactive and the vehicles are known to be generally volatile, measurements of the extent of chemical reaction of the chemicals with the vehicles and the physical behavior of the mixtures will affect their effectivenesses as sterilants. The chemical stability was studied by several qualitative techniques.

1. INFRARED ANALYSIS

Infrared spectra were obtained for each of the five chemicals and each of the six vehicles as well as of all thirty of their binary mixtures. These spectra were measured within 24 hours after mixing. In cases where appreciable time elapsed between mixing and measuring, the specimens were stored with dry ice in an insulated box.

Aliquots of each of the mixtures were placed in linear polyethylene bottles* and were exposed for 19 hours to 58°C in an oven. The spectra of these heated mixtures were also obtained.

*Royal, #24-410 Modified Cylinder Rounds in Linear Polyethylene; Royal, #5-1950 White Polyethylene side-spray hinge caps; Royal, Natural Polyethylene spray tubing.

In some few cases, spectra were obtained by compensation; that is, pure vehicle was used in the compensation cell in a double beam instrument. In most cases, however, adequate results were obtained by using a cell of sufficient thickness (0.1 milliliter) to insure appreciable absorbance by the chemicals.

Table VIII shows the results of comparing the spectra of the individual chemicals and vehicles with those of the mixtures. It is interesting to note that ethylene imine appears to react most strongly with the vehicles though this effect is not confirmed by subsequent spectra of heated samples. It is possible that reactions between ethylene imine and the vehicles reach equilibrium quite rapidly.

In order to determine whether the changes observed at room temperature were true chemical reaction by the chemicals and vehicles rather than possibly effects of an impurity introduced in the mixing operations, infrared spectra obtained after heat treatment were compared with those of the original mixtures. Any reaction between the chemicals and vehicles would not generally be expected to occur rapidly at room temperature. In the case of slow chemical reactions, an amplification of the change in the spectrum would be expected for the heated specimens. Table VIII shows the results of these comparisons. Only the specimens involving ethylene imine and formaldehyde in the mixed solvents appear to involve chemical reaction. The qualitative nature of these tests preclude estimating the extents of chemical reaction.

2. INDEXES OF REFRACTION

Because the infrared spectrometer was unable to accommodate specimens containing appreciable amounts of water or methanol, the comparisons of the freshly mixed and heated samples containing these vehicles were made through the indexes of refraction. Essentially no changes were observed in the methanol mixtures. One might therefore surmise that the extent of chemical reaction was relatively small. In the water solutions, however, all of the chemicals, with the exception of formaldehyde, showed a change in the index of refraction in the heated sample from the original mixture of 0.0018 units. These four chemicals may be presumed to react with the water to some extent.

3. OBSERVATIONS ON STORAGE OF CHEMICAL-VEHICLE MIXTURES

In Table IX, are shown the features of the linear polyethylene bottles and their contents when they were returned from the infrared spectrascopist. That reaction must have occurred in several instances either between the chemical and the vehicle or the combination with the container is apparent. Because the chemical-vehicle combinations used in sterility tests were stored only for brief periods of time, and then under refrigeration, while in the polyethylene containers, the effect of this reaction on the sterilizing effectiveness of the mixture was not likely great. The comments about pure chemicals and pure vehicles are presented in the O column and row of the table.

4. FORMATION OF EMULSION

The following mixtures did not form stable emulsions when passed through a laboratory homogenizer but separated into two distinct phases in the container upon standing for an hour or so.

Epibromohydrin in 2% w/v Tide in distilled water

Formaldehyde in Solvent M-17

Formaldehyde in Solvent M-50

Formaldehyde in Trichloroethylene

With formaldehyde, the nonaqueous phase was used as the liquid sterilant being tested in Phase I but the aqueous phase was used with the epibromohydrin. The following mixtures formed stable emulsions when homogenized.

Epichlorohydrin in 2% w/v Tide in distilled water

Beta-propiolactone in 2% w/v Tide in distilled water

A purer grade of beta-propiolactone, called Betaprone (a product of Testagar & Co., Detroit, Michigan), is soluble in water in concentrations much larger than 5%, but was not available when work started.

5. BETA-PROPIOLACTONE

The extent of solubility of beta-propiolactone in water was studied. To a clean 100 milliliter volumetric flask, one milliliter of beta-propiolactone/Eastman was added. Distilled water was added one milliliter at a time. In the first milliliter of water,

the beta-propiolactone was dispersed readily to form a cloudy liquid. Holding the flask under a running hot-water tap produced a clear solution. When the liquid cooled, it again became cloudy. The same procedures were used in subsequent additions of one milliliter aliquots of distilled water and the same phenomena occurred even after the addition of twenty milliliters of distilled water. In the preparation of 5% v/v beta-propiolactone in water, used in Phase II, the beta-propiolactone dispersed readily into the water in very small droplets as the water was added to the beta-propiolactone in a glass flask. These droplets seemed to coalesce into larger droplets, some of which wet the walls of the flask preferentially to the water phase. The entire contents of the flask were passed through a homogenizer before the liquid was used to treat specimens.

In Table X, information pertaining to the effect of aging and of Tide on the sterilizing effectiveness of 5% v/v beta-propiolactone in vehicle is presented. This information confirms the known fact that beta-propiolactone in water is unstable, particularly so at room temperature and above. The stock of beta-propiolactone was stored at 7-8°C for six weeks and then in the freezer after that. The solutions of beta-propiolactone were stored at 7-8°C.

The information for subject p in Table I of Phase II further illustrates the effect of this instability on the sterilizing effectiveness of solutions of beta-propiolactone. Subjects p and p' were nearly identical. The beta-propiolactone used to treat subject p had been mixed with water about 26 hours prior to use and had been stored at

7-8°C during this 24-hour period. A freshly prepared solution was used to treat subject p'.

On one occasion, the laboratory homogenizer was insufficiently cleaned after having been used to homogenize beta-propiolactone in water. In two days time, the inside mechanism became coated with a sticky green substance which was insoluble in acetone or trichloroethylene but was readily soluble in water.

6. WATER CONTENT

Water content of the 5% v/v ethylene imine in trichloroethylene and of the 5% v/v beta-propiolactone in Solvent M-17 chemical-vehicle combinations (used also in Phase II) were found to be 0.78% w/w and 0.68% w/w, respectively. The water was determined by the Karl Fischer method.

7. RELATIVE VOLATILITY OF SEVERAL CHEMICAL-VEHICLE COMBINATIONS

In Table XI are shown the number of seconds which elapsed after spraying each of the chemical-vehicle combinations on the magnesium alloy strips before the liquid had completely evaporated into the ambient still air. The mixed solvents were particularly volatile and were difficult to maintain in contact with the inoculum for the prescribed exposure times. Only by thoroughly saturating

the insides of the Petri dish with the liquid was the surface of the magnesium alloy strip maintained wet for most of the 90 minutes. The gas phase in the Petri dish was nearly saturated during the entire 90 minutes.

III. PHASE II

A. METHOD

1. STERILITY TESTS, PHASE II'

Each of the fifteen subjects, a, e, f, g, h, i, j, k, k', l, m, n, o, o', and p, (see list of subjects following) were inoculated with from one to five million viable spores of Bacillus subtilis, var niger. The spores were suspended in distilled water and, while so suspended, were deposited as single drops on the surfaces to be inoculated. The volume of the inoculum was metered using a tuberculin syringe.

The inoculum on subjects a, e, f, g, h, i, and j, was placed on the surface of the specimen as a single drop. The inoculum was contained in 0.01 ml. of spore suspension. The location of the several inoculums placed on subjects k, k', l, m, n, o, o', and p, are shown in Figures 1 through 8. On subject o, the inoculums were placed in pin holes, 36, 19, 2; at the base of pins 19, 20 or 20,21 (the inoculum would bridge between the pins); and on the reverse side in the region 19,23.

On subject o', the inoculums were placed in pin holes DD, HH, BB; at the base of pin DD, EE or DD, CC (the inoculum would bridge between two pins) and at the reverse end of pin HH.

While at room temperature, the water was permitted to evaporate from the spores and the subject into the surrounding still air. During this drying operation, the inoculated subjects were lying on a clean, though not necessarily sterile, surface with the inoculated side up

in a clean, though not necessarily sterile, environment, reasonably free from air currents.

Because these subjects were sprayed on all exterior surfaces with the candidate sterilants

a. 5% v/v beta-propiolactone in distilled water
b. 5% v/v beta-propiolactone in Solvent M-17
c. 5% v/v ethylene imine in trichloroethylene
d. 5% w/v formaldehyde in methanol (with about 5% v/v water from the formalin solution)

the specimens were not sterilized nor contained in closed containers before or during inoculation.

Before inoculation, subjects k, k', l, m, n, o, o', and p, were disassembled. The inoculum of one million spores was applied to each of the surfaces which mate (shown in Figures 1-8 inclusive) and thereby became occluded when the subject was assembled. After inoculation, the disassembled subject was dried in the manner discussed above and remained disassembled until after exposed to the candidate sterilants. The inoculum on the electrical connectors subjects o and o', was dried partially under vacuum to insure that spores had an opportunity to reach the bottoms of the pin holes and the surfaces between the pins and the insulation.

In no case were more than five surfaces on any one specimen inoculated.

The spores, which had been supplied by JPL, had been suspended in distilled water at a concentration of 10^9 cells/milliliter in Phase I. In this suspension, the concentration of spores, which

demonstrate viability when incubated on Trypticase soy agar, was measured. The volume of the 10 to 1 dilution of the stock suspension required to deposit an inoculum of one million demonstrably viable spores was 0.01 milliliters.

After washing, all spore handling equipment was sterilized in saturated steam at 120°C for 1 hour before use or re-use.

When the inoculums were sufficiently dried, each of the four candidate sterilants were applied to a fresh inoculated specimen. The specimens were prepared in triplicate. For subjects a, e, f, i, and j, each set of triplicates and the bacteriostasis control were placed inside a sterile petri dish immediately after the sterilant had been sprayed on the specimen. During spraying, the specimen was suspended vertically while it was held by forceps. The spraying took place in a quiet zone in a hood and the petri dish remained in the same quiet zone until after it received the specimen. The specimens remained horizontal during the subsequent 24 hour storage period in the petri dishes.

Subject h was placed on the bottom of a 10-ml. beaker and was sterilized by exposure to 125°C for 24 hours, while in a baby food jar with the cap in place. The inoculum was placed on the top surface of the grease and was permitted to dry with the lid sitting loosely on the jar. The candidate sterilant was applied to the inoculum by spraying. The caps were tightened on the jars to make hermetic seals.

Subjects g, k, k', l, m, n, o, o', and p were dipped in the candidate sterilant. In addition, all holes such as screw holes, connector socket holes, etc., which were inoculated were flushed by means of a syringe and needle. The dip was quick and the subject was assembled immediately. Then the exterior surfaces were sprayed with the sterilant. The assembling and the spraying operations took place in a hood. Within five minutes after being sprayed, and actually as soon as possible, the specimens were moved to the inside of a sterile enclosure, Figure 9, where they were placed inside individual,sterile,plastic bags. The tools, gloves, and hands used for assembling these objects were not sterilized. The plastic bags and their contents were sterilized however. The gloves and tools used in assembly were clean in order to avoid cross mixing of candidate sterilants.

The sterile enclosure was illuminated with a Westinghouse Sterilamp #782L-30. The ultraviolet light did not reach the occluded surfaces on the specimens where the inoculum was placed. The light did, however, serve as an air sterilant and reduced the number of bacteria residing on the outside of the plastic bags. The effectiveness of this technique for eliminating the introduction of airborne bacteria into the bags was measured. After the specimen was placed in its own individual, sterile plastic bag, the bag was closed by folding and crimping with a rubber band. The bags were placed in a clean, though not necessarily sterile, environment at room temperature for 24 hours.

These plastic bags had double walls consisting of two separate 0.001-inch thick polyethylene films and were 12 inches wide by 20 inches long. These double-walled polyethylene bags contained ethylene oxide for 48 hours prior to receiving the specimens. Into each bag were placed the tools for disassembling, the jar of sterile water, and a jar containing 2.30 ml. of a mixture of 0.10 milliliters of distilled water and 2.22 ml. of liquid ethylene oxide. The amount of water was designed to insure that the water would exceed that required to produce 30% relative humidity.

The ethylene oxide and water were mixed in a chest packed with dry ice. Unless the mixture was warmed occasionally, a solid phase would form, indicating the formation, possibly, of an hydrate. The same thing has happened with this same mixture when it was immersed in a water-ice bath. The mixture never got much below 0° C, before the solid formed. This solid hydrate might provide a convenient method of handling ethylene oxide for sterilization purposes.

The effect of the residual ethylene oxide on spore viability was measured and found to be negligible. The effectiveness of the bag and tool sterilization procedures was measured.

At the end of 24 hours, the specimens of subjects a, e, f, i, and j were lifted out of the petri dishes and into individual sterile baby food jars containing 50 milliliters of sterile distilled water. The specimens were handled with sterile forceps only.

The jars containing the specimens in the water were immersed for one minute in an ultrasonic field. Subject h in its beaker was also transferred to a container of 50 milliliters of sterile distilled water. All these transfer operations took place in the sterile enclosure, Figure 9, in the presence of filtered air, but without UV irradiation.

At the end of the 24 hours, components k, k', l, m, n, o, o' and p were disassembled while still enclosed in the bag. The components were all dropped into the water in the jar and the jar was closed tightly. Then, and only then, was the bag opened and the jar removed. The tightly closed jar containing the subject in the water was immersed for one minute in water in an ultrasonic field.

Within a few minutes after being removed from the ultrasonic field, 1.4 milliliters of the liquid in the jar was withdrawn into a clean sterile 5-ml. glass syringe. A 0.2-milliliter aliquot was expelled from the syringe onto the surface of a sterile Trypticase soy agar plate. A second 0.2- milliliter aliquot was expelled from the syringe onto the surface of a second sterile Trypticase soy agar plate and the remaining one-milliliter aliquot was expelled into nine milliliters of sterile distilled water inside a sealed vial which had been sterilized with the water in it. The sterile Trypticase soy agar plates used in all assays described in this proposal, were obtained from Hyland Laboratories, and the sterile water jars and vials from Morton and Converse.

Five milliliters of the contents of this vial were withdrawn into the syringe and then returned to the vial. Four additional withdrawals and returns were made. One and four-tenths milliliters of the contents of the vial were then drawn into the syringe. A 0.2-milliliter aliquot was expelled onto the surface of a third sterile Trypticase soy agar plate. A second 0.2-milliliter aliquot was expelled onto the surface of a fourth sterile Trypticase soy agar plate and the remaining one milliliter aliquot was expelled into nine milliliters of sterile distilled water inside a second closed sterile vial. The process of mixing, withdrawing an aliquot and expelling onto the sterile Trypticase soy agar plates was repeated in a manner identical with that of the first vial.

From the second vial only 0.4 milliliters were withdrawn because only the two sterile Trypticase soy agar plates, numbers 5 and 6, received one milliliter of liquid each. By nutation, the liquid on the surfaces of all the Trypticase soy agar plates was distributed as uniformly as possible.

Each subject was processed in this identical manner to produce six test plates except for the bacteriostasis controls where plates 5 and 6 were not prepared. For those plates prepared using subjects which had never been inoculated, the bacteriostasis controls, an aliquot of the spore suspension sufficient to produce approximately 100 colonies on each plate, was added to the contents of the corresponding jar or vial. The plates were

incubated at 37 ± 2°C for seven days. The number of colonies which developed on each of the plates was counted and recorded in Table I of Phase II.

2. COMPATIBILITY TESTS, PHASE II"

The subjects used in Phase II" were prepared for treatment with the candidate sterilants in the same manner as they had been prepared for the Phase II' studies. To the extent possible, the analogous operations in Phases II' and II" were performed concurrently.

The method used to apply the sterilant to each of the specimens is indicated in the following list of subjects. Subjects k, l, m, n, and p had considerable amounts of cutting oil and metal powder on them resulting from machining operations. They were rinsed in Solvent M-50 and dried before use in the measurements described here for Phases II' and II". All other subjects were used in the as received or as prepared state.

Subject a	Strip of sheet magnesium alloy AZ 31 B approximately 1-1/4 inches x 3 inches x 0.016 inches with a machined surface finish 125 microinches rms or better, and a Dow 7 surface treatment. Each specimen was sprayed with the candidate sterilant and placed in a closed petri dish and left there for 24 hours. While being sprayed, the specimen was held vertically by forceps.
Subject b	Temperature control surface, white silicone paint, on one side, uncured, on magnesium, in three shapes 6" x 6", 2" x 2", and 15/16" diameter.

Sterilant was applied by spraying while the specimen was held in an aluminum rack. The rack and the specimens were placed inside a polystyrene box for at least 24 hours.

Subject c — Temperature control surface, black silicone paint, on one side, uncured, on magnesium, in three shapes - 6" x 6", 2" x 2", and 15/16 diameter.

Sterilant was applied by the same technique as was used for subject b.

Subject d — Temperature control surface, gold plated, in three shapes 6" x 6", 2" x 2", and 15/16 diameter.

Sterilant was applied by the same technique as was used for subject b.

Subject e — Teflon strip, approximately 1-1/4 inches x 3 inches x 0.010 inches cut from a sheet of teflon film.

Sterilant was applied by dipping the specimen into sterilant and storing it in a petri dish as was done for subject a.

Subject f — Stycast 2340M strips approximately 1/4 inches x 1/4 inches x 2 inches cut from a sheet cast on teflon film in accordance with manufacturer's directions.

Sterilant was applied by same technique as was used for subject e.

Subject g — Epoxy-fiberglass terminal board - Specification MIL-P-18177-GEE approximately 1 - 1/4 inches x 1/2 inch x 3 inches.

The specimens were flushed with sterilant. A syringe and needle used to apply sterilant to all surfaces. The specimen was sprayed and then placed in a polyethylene bag where it remained for 24 hours.

Subject h — Silicone grease, General Electric Co., Versilube G-300(Chlorophenyl methyl silicone fluid with a lithium soap and antioxidant.) One gram of grease was spread over the bottom of a clean ten-milliliter beaker. The beaker and its contents were exposed

to dry air at 125° C for 24 hours. When again cool, five milliliters of sterilant were poured gently down the inside of the beaker. The beaker was swirled gently for three minutes and the sterilant then decanted from the grease.

Subject i — Silicone rubber strip, Specification AMS-3302B, Kirkhill Rubber Company, approximately 1/4 inch x 1/4 inch x 1 1/4 inches.

Sterilant was applied by the same techniques as were used for subject e.

Subject j — Butyl rubber strip, Stillman Rubber Co., SR 613-75, approximately 1 1/4 inches x 3 inches x 0.12 inches.

Sterilant was applied by the same techniques as were used for subject e.

Subject k — Screw into lock nut and conducting flange, Figure 1.

Sterilant from a syringe needle flushed out interior surfaces before disassembled components were dipped in the sterilant. After treatment, the specimens were placed in a polyethylene bag where they remained for 24 hours.

Subject k' — Screw into lock nut and conducting flange, Figure 2. Sterilant was applied to specimen in the same manner as was used for subject k.

Subject l — Screw into insert, Figure 3.

Sterilant was applied to specimens in the same manner as was used for subject k.

Subject m — Dowel pin press fit and screw into tapped hole, Figure 4.

Sterilant was applied to specimens in the same manner as was used for subject k.

Subject n — Cable clamp, Figure 5.

Sterilant was applied to specimens in the same manner as was used for subject k.

Subject o — Cannon electrical connectors, DOM 508 NM 1 with DOM 50P NM 1, Figure 6.

Sterilant from a syringe flushed out those pin holes which were used in the electrical resistance measurements. The disassembled specimen was dipped in the sterilant and then assembled and placed in a polyethylene bag where it remained for 24 hours.

Subject o' Bendix pygmy connectors, PT 00A 22 55S with PT 06A 22 55P, Figure 7.

The sterilant was applied to the specimens in the same manner as was used for subject o.

Subject p Shaft fit with O-ring, Figure 8.

Sterilant was applied in the same manner as was used for subject k.

Subject q 6061 aluminum sheet, with standard mill finish, to which strip coat TEC-734-P/Tec Chemical had been applied.

Sterilant from a syringe needle flushed the specimens on all surfaces. The specimens were stored in petri dishes for 24 hours after exposure to the sterilants.

All subjects which were to be disassembled before treatment with the candidate sterilants were disassembled and treated with the candidate sterilant. Special additional replicates of all specimens of subjects b, c, and d, were prepared by JPL. All subjects were treated with the candidate sterilants.

After applying sterilant, subjects k, k', l, m, n, o, o', and p, were assembled promptly (within 5 minutes) and received by spraying a second application of sterilant which covered all exposed surfaces. All subjects were then stored at room temperature in double-walled polyethylene bags for at least 24 hours. Duplicates of each subject treated with each candidate sterilant were prepared and two specimens of each subject, which had not been exposed to any of the sterilants, were set aside. A total of eight specimens of each subject, except b,

c, and d were treated with candidate sterilants for the purposes of Phase II".

A total of 8 specimen of each shape for subjects b, c, and d, plus an additional 8 specimens of the 2 inch by 2 inch square shape were treated with the candidate sterilants in order to supply specimens to JPL for reflectance measurements.

After 24 hours, each of the ten specimens of each subject was inspected and the significant features of its appearance, particularly differences attributable to exposure to the sterilants were noted.

a. Change in Weight

After preparation, subjects b, c, e, f, g, i, and q were weighed on an analytical balance to the nearest 0.1%. After exposure to the sterilants and storing for 24 hours they were weighed again so that a change in weight could be detected.

b. Change in Dimension

The thicknesses of subjects e, i, and j were measured using a micrometer caliper both before and after exposure to the candidate sterilant.

c. Tackiness

A dry swab of absorbent cotton wrapped around a wooden stick, was placed firmly against each specimen

of subjects b, c, e, f, g, i, and j. Whether or not any cotton adhered to the specimen, whether any of the specimen adhered to the cotton, and whether any visible indentation was left in the specimen was noted.

d. Optical Properties

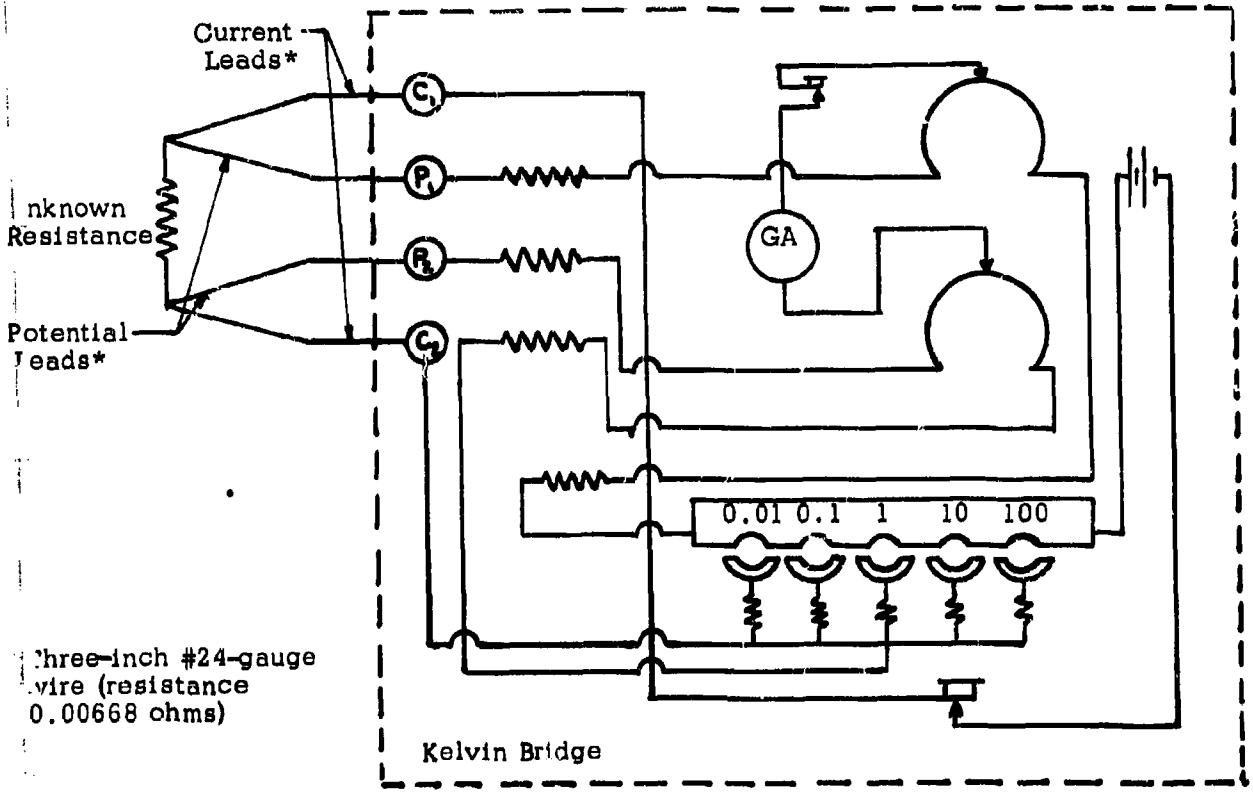
When the paper tissue, in which they had been wrapped before exposure to the sterilants, was removed from the white surfaces, it seemed to adhere to every specimen and a pattern of the paper was left in the white paint. Sterilants B and C evaporated extremely rapidly making it difficult to maintain the entire surfaces of subjects b, c, and d, wet when the specimen was finally at rest inside the bag. Sterilant A failed to wet any of the temperature control subjects and was therefore deposited as a fog on the surface. In some instances, the fog droplets coalesced into large droplets which remained in place on the specimen while it was in the bag. Sterilant D wetted the black and gold surfaces but did not wet the white surfaces.

The JPL replicates of temperature control surfaces, subjects b, c, and d, were packaged in polystyrene boxes and returned to JPL without applying any tests (appearance, weight, or tackiness) so that they would be suitable for measuring changes in reflectance.

e. Electrical Measurements

i. Contact Resistance (Subjects k, o, and o'and k')

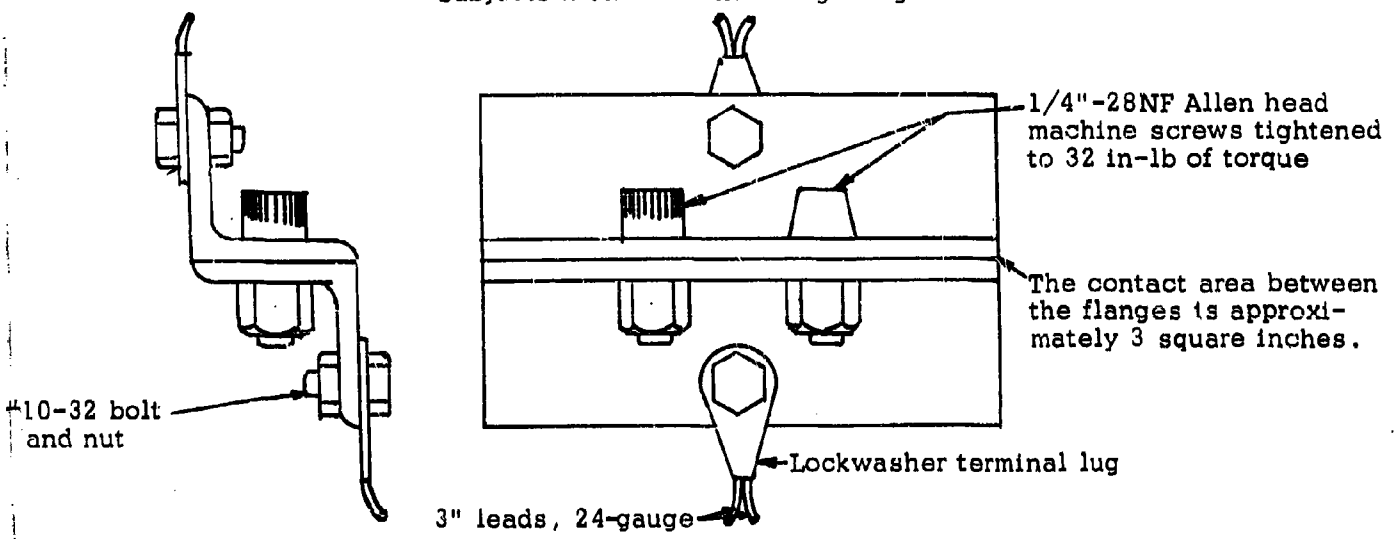
Using a Leeds and Northrup Kelvin Bridge, Model No. 4286, the contact resistance of subjects k, k', o, and o' was measured both before and after exposure to the sterilants. The current leads were separate from the potential leads in accordance with the specifications in the contract. The wiring diagram for the measuring circuit is shown in the following sketch:



The manufacturer reports the accuracy of the Kelvin Bridge as ± 2%

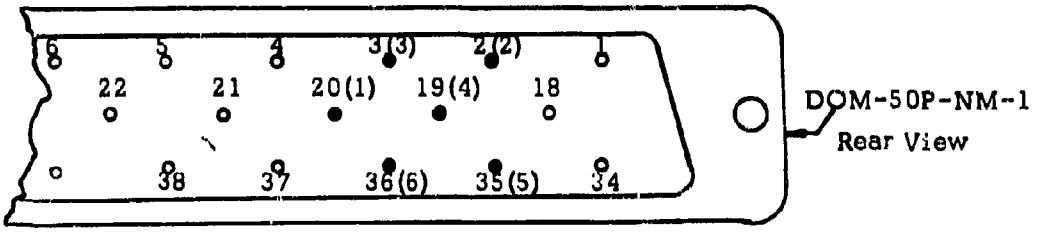
The wiring details for each of the four subjects are shown in the following sketches.

Subjects k and k' conducting flanges



Subject o, Cannon electrical connector, DOM-505-NM-1 with DOM-50P-NM-1

Contact resistance was measured for pin numbers 2, 3, 19, 35, and 36. The grouping of these pins is indicated in the sketch below:

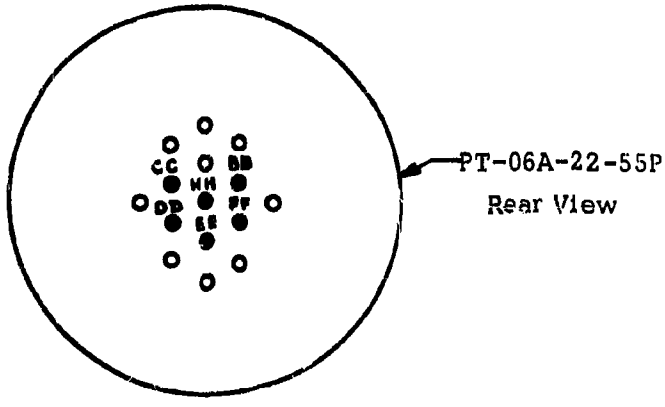


In the tabular information presented elsewhere in this report, the pins have been designated by the numbers in parenthesis.

Two three-inch leads of 24-gauge wire (resistance: 0.00668 ohms) were soldered to each of the designated pins on the plugs and on the sockets. These wires constituted the separate current and potential leads. Spade terminals were used on the current leads and pin terminals were used on the potential leads.

Subject o', Bendix pygmy connector, PT-00A-22-55S with PT-06A-22-55P

Contact resistance was measured for pins identified on the connector as BB, FF, HH, CC, and DD. The grouping of these pins is indicated in the sketch below:



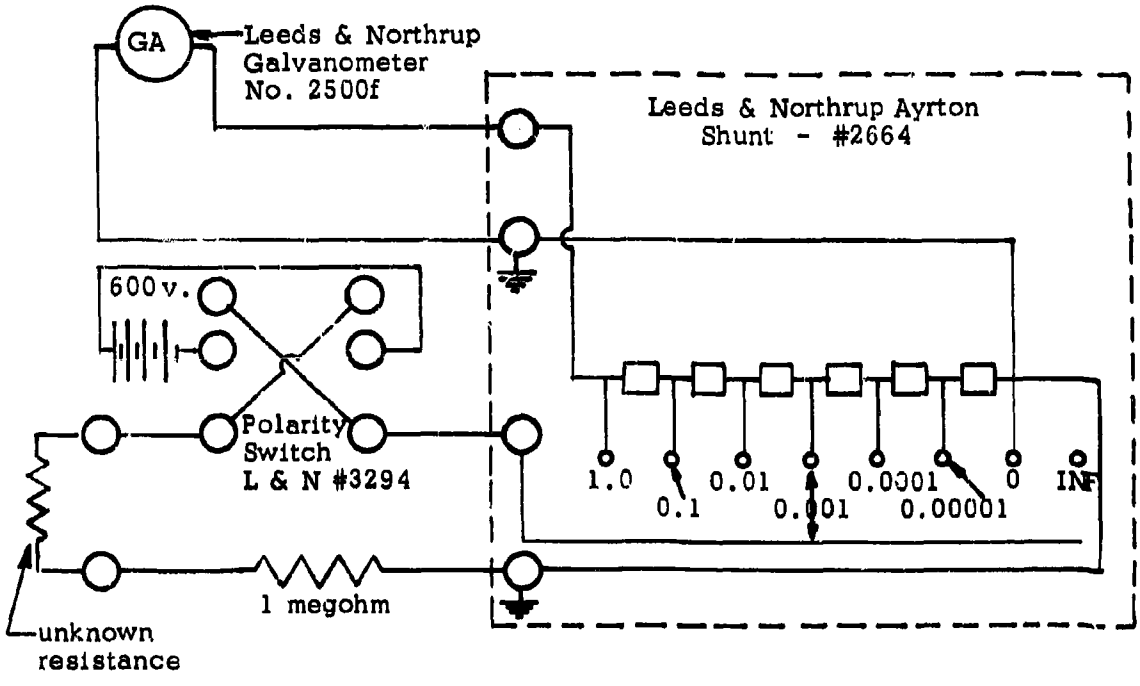
In the tabular information presented elsewhere in this report, the pins have been designated by the following numbers: 1. (EE), 2. (CC), 3. (DD), 4. (HH), 5. (BB), and 6. (FF).

Two three-inch leads of 24-gauge wires (resistance: 0.00668 ohms) were soldered to each of the pins 1, 2, 3, 4, 5 and 6 on the plugs and on the sockets. These wires

constituted the separate current and potential leads. Spade terminals were used on the current leads and pin terminals were used on the potential leads.

ii. Insulation Resistance (Subjects o and o')

The insulation resistance between pin No. 1 and five other pins for each of these subjects was measured using the following circuit:



For subject o the insulation resistance was measured between pin number 20 and pins numbered 2, 3, 19, 35, and 36. The grouping of these pins is indicated in the sketch of subject o in the discussion of contact resistance measurement.

For subject o' the insulation resistance was measured between pin EE and pins BB, FF, HH, CC, and DD. The grouping of these pins is indicated in the sketch of subject o' in the discussion of contact resistance measurement.

The potential source was two Burgess #493 300-volt dry cells in series. The standard resistor was one megohm with an accuracy of 1%. The manufacturer reports the accuracy of the Ayrton Shunt to be $\pm$ 0.1% and the sensitivity of the galvanometer to be 0.0001 microampere per millimeter of deflection at a distance of one meter.

Because the resistance of the unknown is related to galvanometer deflection by

$$R = 10^6 \frac{d - d_1}{d_1} \quad *$$

the voltage of the batteries does not affect the resistance measurement directly. It does affect the sensitivity of the circuit to measure large resistances, however.

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*in the above equation, R is the resistance of the unknown, d is the deflection of the galvanometer for a direct short across the unknown resistor and d_1 is the deflection of the galvanometer for the unknown resistance.

f. Strippable Coatings

The strip coat was applied to subject q by four successive immersions of the aluminum strip in the liquid coating bath. After all other tests on the subject q specimens were completed, the strip coat was partially peeled away to observe how cleanly it separated from the aluminum strip. Except for one spot about one millimeter square on one of the Sterilant A replicates, all coats peeled away cleanly.

g. Solubility of Silicone Grease

In all tests of specimens of subject h, one gram of grease was spread over the bottom of a ten-milliliter beaker. Five milliliters of the candidate sterilant were poured gently down the side of the beaker. The beaker was swirled gently for three minutes and the sterilant then decanted onto a filter paper in a Buchner funnel to which suction had been already applied. The filter and the suction were so chosen that five milliliters of candidate sterilant, not exposed to grease, would pass through it in ten seconds. The filtrate was collected in a previously weighed flask and was evaporated to dryness at a temperature not over 40° C. The flask containing the residue was weighed and the weight of the residue recorded to the nearest milligram. Even after drying

for days and dessication over Drierite a liquid remained with the Sterilant A residue. This liquid was apparently beta-propiolactone or one of its products.

B. RESULTS AND DISCUSSIONS

1. STERILITY TESTS, PHASE II'

In Table I of Phase II are presented the numbers of colonies of B. subtilis, var. niger which developed on the Trypticase soy agar plates described in the Phase II' Methods section of this report. Table I also presents the number of colonies observed on the bacteriostasis control plates and on the Control 2 plates. The Control 2 plates indicate the effectiveness of the viable spore recovery procedures. As in the case of Table I of Phase I, the numbers of colonies of contaminating microorganisms which developed on the plates are shown in parentheses. The tests reported in Table I were performed in such a manner that they could be divided into four batches which were equally representative of a total set of conditions. Subject k' and p', however, were tested last and consequently have no batch numbers.

Subject p' represents a repeat test in which subject p was exposed to a freshly prepared emulsion of beta-propiolactone in distilled water. Subject a specimens were exposed to beta-propiolactone in water within 2 hours after it had been mixed. Subject l was exposed to the same mix of sterilant within 4 hours after mixing and subject p approximately 26 hours after mixing. The data presented for subject p illustrate the effect of instability of aqueous solutions of beta-propiolactone on sterilization efficiency.

Subject h was exposed to beta-propiolactone in water approximately four hours after being mixed. In all other cases, the beta-propiolactone in water was used within two hours after being prepared.

Table II presents the total number of colonies of B. subtilis, var. niger observed on the plates for all dilutions, plates, and specimens and indicates the corresponding sterilant, batch number, and subject letter for each total number. In Table III, an analysis of variance of the data of Table II is presented. This analysis of variance indicates that both the subjects and the sterilants have an effect on the number of colonies which develop greater than that which can be assigned to batch differences or to random uncontrolled factors. The significance of these effects of subject and of sterilant is not great with respect to the effect of the random uncontrolled factors.

Table IV presents the total number of colonies of contaminating microorganisms which developed on the agar plates for all dilutions, replicate plates, and replicate specimens, and has designated a corresponding subject, batch, and sterilant for each. In Table V, an analysis of variance in the number of colonies of contaminants, from Table IV, is presented. This analysis indicates that the number of colonies of contaminants varied quite significantly from day to day in the sterility test procedures but was not related to the subject or to the sterilant.

Table VI presents the number of colonies of B. subtilis, var. niger appearing on bacteriostasis control plates, so that the effect of possible bacteriostasis might be made more apparent. Bacteriostasis appeared to occur in the cases of subject e exposed to beta-propiolactone in water and to formaldehyde in methanol in the case of subject i exposed to formaldehyde in methanol, and in the case of subject l exposed to ethylene imine in trichloroethylene. In Table VII, the number of colonies on the bacteriostasis control plate is subjected to an analysis of variance. The analysis indicates that the differences in bacteriostasis were due far more to differences in technique between batches than to any of the other factors. From looking at column T_* in Table VII, it is apparent that the measurements performed in Batch IV had an exceptionally low frequency of colonies appearing on the bacteriostasis control plates. There is always the possibility of human error having produced a spore suspension for use in the bacteriostasis control which was substantially lower in spore concentration than that which was desired. The reason for the low counts in Batch IV is, frankly, unexplained. The counts were, however, uniformly low for all subjects and sterilants in Batch IV.

In Table VIII, the subjects are ranked by the percent of the viable spores recovered from inoculated specimens which had not been exposed to the sterilants. The recovery of spores from subject a was substantially smaller in the Phase II' work than it had been in the Phase I measurements. The surface of k' was similar to that of a but

the spore recovery rate was higher. Table VIII indicates the difficulty of demonstrating absolute sterility by any procedure which involves rinsing possibly contaminated objects and preparing plates from the rinse liquid. It also indicates that even with ultrasonic scrubbing the variability in the fraction of the spores recovered is quite large.

2. COMPATIBILITY TESTS, PHASE II"

a. Change in Appearance

Many of the subjects changed appearance after exposure to the candidate sterilants. A verbal description of this change in appearance is difficult but has nevertheless been attempted in Table IV. Formaldehyde in methanol frequently left a hard white deposit of paraformaldehyde. Otherwise, this candidate sterilant seemed to effect the appearance somewhat less than did the other sterilants. None of the candidate sterilants was without effect on all subjects.

b. Change in Weight

Table X presents the weights of several of the subjects both before and after exposure to sterilants. While the susceptibility of the subjects to change in weight was by no means uniform an analysis of variance of the relative change in weight has been

calculated and presented in Table XI. The potting compound, subject f, appears to absorb substantial amounts of most of the sterilants, while the stripcoat appears to lose material into the sterilant which subsequently evaporates. The effect of the nature of the subject on relative change in weight is highly significant when compared with the effect of random uncontrolled factors.

c. Change in Dimension

Change in weight of a subject is not necessarily indicative of the extent of volume dilation. In Table XII are recorded measurements of the thicknesses of three of the subjects both before and after exposure to the sterilant. An analysis of variance of the percent change in dimension of these subjects is presented in Table XIII. This analysis shows that the subject e was affected much less by exposure to sterilants than were rubber subjects i and j and that formaldehyde in methanol was somewhat less conducive to changing dimensions than were the other sterilants.

d. Tackiness of Subjects

Excluding subject h, none of the subjects were tacky with respect to the cotton swab test either before or after treatment. By exercising one's imagination, temperature control surfaces b and c might have had a slight tackiness by this test.

Tackiness, however, was observed in other ways. Subject q stuck to the glass plate after it had been exposed to sterilant B. Subjects o and o' felt sticky on all surfaces after exposure to sterilant B. There was an oily deposit on subject o' after exposure to sterilant C. Subject q was tacky to touch after exposure to sterilant D and subject m had a gummy deposit after exposure to sterilant B.

e. Contact Resistance

In Table XIV, the contact resistances of subjects k, k', c, and c', have been recorded. The "after treatment" heading does not apply to the Control 1 and Control 2 specimens. The observations recorded for these specimens under this heading really represent replicates of the measurements recorded in the "before treatment" columns. Here "Control 1" and "Control 2" have meanings different from those used in discussing sterility tests. Here these terms refer to untreated specimens. The contract specified that the after-treatment measurements would be made within 3°C of the temperature at which the measurements before treatment were made. The relative humidity of the air in which the subjects were measured did not change more than 10 percent between the before and after treatment measurements. The contact resistances were measured promptly after being removed from the bag after

24 hours storage. It would not be reasonable to presume that the specimens were all in equilibrium with the air with which they were in contact during measurement. They were not far from equilibrium, however, because the air in the room was not greatly different in either temperature or relative humidity from that which was in the bags. In Table XV are recorded the relative changes in contact resistance for subjects k and k'. From these relative changes in resistance an analysis of variance in the relative change in contact resistance of these subjects was calculated and recorded in Table XVI. This analysis of variance indicates that the difference between subjects k and k' were highly significant when compared with the effects of all other factors either controlled or uncontrolled. The different sterilants did not manifest a significant difference on the contact resistance of these subjects. The difference between replicates may be attributed in part to the fact that the lock nuts exerted considerable friction against the screws. Consequently, the uniform tightening torque did not produce a uniform contact pressure in the contacting plane. In Table XVII are recorded the contact resistances for subjects o and o' and the analysis of variance in these contact resistances is recorded in Table XVIII. The analysis of variance indicates that the differences in contact resistances of subjects o and o' was substantially greater than the differences produced by treatment with the sterilants.

Exposure to the sterilants did have a substantial effect on the contact resistances of these subjects. The differences among the effects of the sterilants were not, however, particularly large.

In Table XIX is recorded information about the reproducibility of the contact resistance measurements.

f. Resistance of Insulation

Table XX reports the basic data obtained in measuring the electrical resistance of the insulation of subjects o and o'. From this information the logarithm of the electrical resistance of the insulation was calculated and recorded in Table XXI. The measurements before and after exposure to the sterilants were made within 3°C and 10 percent relative humidity of each other. Exposure to sterilant A, beta-propiolactone in water, reduced the logarithm of electrical resistance by fifty percent. This loss in resistance may be due to the formation of a conducting film of ionic beta-hydracrylic acid in water from the hydration of beta-propiolactone. In the case of subject o' this effect was large where it did not appear in the case of subject o. Table XXII presents an analysis of variance table for the data in Table XXI. Nearly all of the variation is accounted for by the effects of differences between subjects, the effects of treatment, and the effects of differences

among the sterilants. All three of these factors produce significant effects on the electrical resistances of the insulators.

g. Solubility of Lubricant

In Table XXIII are reported the basic observations about the solubility of Versilube G-300, silicone grease, in the four candidate sterilants. Table XXIV presents the corresponding analysis of variance. The effect of sterilant on the solubility was highly significant. In the sterilants actually exposed to subject h, the beta-propiolactone was present in an amount of 0.25 milliliter. This substance apparently extracted much material from the grease or reacted with components of the grease in some way to make them water soluble. Solvent M-17 and trichloroethylene dissolved some of the grease, as might be expected. The residue from the formaldehyde sterilant extraction appears to be simply paraformaldehyde.

h. Surface Wetting by Candidate Sterilants

In Table XXV has been recorded information about the ability of the sterilants to wet each of the subjects. This property of the sterilant can be expected to have a significant effect on a sterilizing efficacy.

3. OTHER TESTS

a. Effects of Concentration, Alcohol and Exposure Time on Sterilizing Effectiveness of Formaldehyde Sterilants and Ethylene Imine Sterilants

The information in Table XXVI was developed for the purpose of exploring the effects of concentration of formaldehyde and the molecular weight of the vehicle on the sterilizing efficacy of formaldehyde in alcohol sterilants. In these tests the inoculum was placed on magnesium alloy strips which had been used at least once before and which had been autoclaved at least once. The baby food jars containing the water for rinsing the inoculum off the specimens were prepared in our laboratory and thus did not have the degree of control which is attained by a mass producer of such items.

The glassware had been used in the measurements in Phase I. There is a distinct possibility that an error in technique in the case of the second replicate of methanol containing 5% w/v formaldehyde. The results for isopropanol solutions appear somewhat erratic but suggest that isopropanol may not be as satisfactory a vehicle as methanol, a result somewhat at variance with the recorded experience of other investigators[4], and that its use delays the germination of the spores on Trypticase soy agar. The strip which was exposed to isopropanol was still wet when it went into the rinse jar and possibly contributed to the bacteriostasis. Table XXVII shows the effect of changing the

molecular weight of the vehicle in ethylene imine and alcohol mixtures on the sterilizing effectiveness of these mixtures and of exposure time on the sterilizing effectiveness of ethylene imine in methanol. Exposure times as short as 5 minutes appear to be substantially less effective as those of 90 minutes.

Because the liquid evaporates so rapidly from some of the subjects there is always the possibility of a significantly different exposure to sterilant for two different objects in the same Petri dish. Formaldehyde at room temperature and in those concentrations designated in Table XXVI cannot be expected to sterilize the indicated inoculums when it is present only in the gas phase.

h. Absolute Viability Tests

When the specimen of subject 1, exposed to formaldehyde in methanol, was transferred aseptically from the rinse jar to a bottle of sterile Trypticase soy broth/Hyland and then incubated at 37°C under aerobic conditions, the broth became turbid with B. subtilis, var. niger cells. This difference between earlier measurements on the sterility of subject 1 and these results is not surprising when the low spore recovery rate for subject 1 is considered. In Table XXVIII are reported the results of further studies of absolute viability of four of the subjects to the candidate sterilants. These results indicate the insufficiency of the information

in the earlier tables to demonstrate that any of these sterilants will produce an absolutely sterile surface. Each of the subjects was treated with the candidate sterilants in the usual manner and was held for 24 hours in a sterile Petri dish. It was then transferred aseptically to a bottle of sterile Trypticase soy broth and incubated aerobically for seven days at 37°C. The bottles which contained microorganisms were turbid on the seventh day. The identity of the microorganism was established by its production of an intense orange pigment and by its Gram-staining characteristics. In evaluation of the data in Table XXVIII, it is useful to remember that not all potentially viable spores will germinate in Trypticase soy broth and if they would, not all would germinate in seven days. Germination delay of eighteen months has been reported in the sterilization literature[4].

c. Effect of the Volume of Sterilant and Size of Inoculum on Sterilizing Efficacy

Table XXIX illustrates the effect of the volume of sterilant and the size of the inoculum on sterilizing efficacy using several candidate sterilants. The data on formaldehyde and methanol is highly erratic or else implies that there exists an optimum concentration of formaldehyde in methanol, in the gases over the spores to be sterilized, for achieving sterility. In one of the authors' experience with gas sterilization the concentration

of formaldehyde in gas phase required to sterilize polystyrene is substantially different from that required to sterilize polyvinyl chloride. The difference between the results in this table and those reported earlier require more explanation than is available in the information at hand. The physical and chemical behaviors of formaldehyde solutions are complicated.

The great effectivenesses of both beta-propiolactone in distilled water and in 2% w/v Tide in distilled water preclude deciding which is to be preferred in sterilizing teflon. Teflon was selected for this test because of the great difficulty in finding materials which will wet its surface. Tide was considered in Phase I because of its surface active properties. Tide, however, leaves a deposit on the surface on which its solution has been applied.

The tests whose results are presented in Table XXIX were designed to compare two types of beta-propiolactone in two vehicles. The differences among these sterilants were not perceptable among the results.

In most tests pertaining to Table XXIX the inoculum was placed on a teflon strip. In the earlier tests using formaldehyde, the inoculum had been on a magnesium alloy strip with a Dow 7 surface treatment.

Table XXIX clouds the understanding of the sterilizing mechanism for formaldehyde. The sterilant was placed directly on the inoculum. The inoculum was on a strip in a Petri dish. The sterilant was 5% formaldehyde in methanol. The bacteriostasis control was prepared by adding to the rinse water for the strip, after removing an aliquot for plating, the indicated number of spores. The expected plate count for the 10^4 bacteriostasis inoculum was 100 and for the 10^6 specimen inoculum 10^4.

d. Ethylene Oxide Sterilization of Polyethylene Bags

Table XXX presents data to support the use of an ethylene oxide process in sterilizing the double-wall polyethylene bags and their contents. In the table the first strip was a filter paper strip bearing one million spores of B. subtilis, var. niger. After exposure to ethylene oxide it was transferred to sterile water and rinsed in the usual manner. The bacteriostasis control was prepared by inoculating the water in the rinse jar, after the aliquot for plating had been removed, with enough spores to produce plate counts of approximately 100. The second strip of filter paper bearing one million spores was placed in the bag as the first strip was removed, 48 hours after the strip and ethylene oxide had been placed in the bag. The results for the second strip provide information about the ethylene oxide remaining

in the bag. The number of colonies reported represents the $1:10^2$ dilution of the rinse water. The first strip represents the $1:10^0$ dilution of the rinse water. This information supports the assumption that only negligible amounts of ethylene oxide remain in the bags at the end of 48 hours.

In each of three different double-walled polyethylene bags, a Taylor dial relative humidity gauge was placed. The bag sterilization process was applied to each bag. The relative humidities indicated on the gauge are shown in Table XXXI. Because the gauge was calibrated for water vapor in air, these readings are in error by a small undetermined amount. The Table does confirm, however, that the relative humidity in the bags was high enough for sterilization to take place.

The information presented in Table XXXII was developed from early dry runs on the bag technique used in Phase II. Because ethylene oxide was not then immediately available, Cryoxcide was used. Comparative tests of ethylene imine vapor in the same concentrations and under the same conditions were run because it was convenient to make such tests at this time. Our interest in ethylene imine as a gas sterilant arose from the need for a demonstrably effective method for sterilizing the inside of the bags before the start of Phase II measurements. We chose to run the experiments in parallel,

even though some may have been redundant, in order to reduce the need to make experiments in series. The results of the ethylene imine tests are presented in Table XXXIII.

In each of the bags used, in the aforementioned tests, a small filter paper disk, bearing 0.015 milliliters of distilled water, was placed in the bag with the liquid sterilant, ethylene imine or Cryoxcide, to raise the relative humidity to about 30%. The average molecular weight of the Cryoxcide was assumed to be 112.4. The temperature was 25°C and the amount of Cryoxcide and of ethylene imine used was 10 grams in a previously empty polyethylene bag, 12 inches by 20 inches. Table XXXII and XXXIII show the effect of Cryoxcide and of ethylene imine on B. subtilis, var. niger spores residing on several types of objects. In one case, the cap on the baby food jar did not appear to be on tightly enough to prevent leakage of the sterilizing gas into the inside of the baby food jar.

e. Use of Ultraviolet in Maintaining a Sterile Field

Table XXXIV is submitted to support the use of the ultraviolet light (see Figure 9) in controlling the contamination introduced into the bags during insertion of the treated specimens.

The 10^6 spores of B. subtilis, var. niger were dried on specimens of subject g and on a piece of expanded polystyrene from a disposable plastic cup. The inoculum was placed against the Vycor envelop of the ultraviolet lamp during the exposure. The spores were recovered and plated in the manner described previously for use in Phase II'.

While ultraviolet is slow in killing spores, it does kill them. The effectiveness of the ultraviolet lamp in maintaining air sterile depends upon the effectiveness of the filtration of the incoming air supply. Table XXXV presents the number of colonies observed on fallout plates which were in the ultraviolet chamber during the indicated operations with the plastic bags.

III. REFERENCES

1. C. R. Phillips. American Journal of Hygiene, 50, 280 (1949).

2. The Pharmacopeia of the United States of America, Sixteenth revision. Mack Publishing Co., Easton, Pa., (1960).

3. C. A. Bennett and N. L. Franklin. Statistical Analysis in Chemistry and the Chemical Industry. Wiley, New York, (1954).

4. G. F. Reddish. Antiseptics, Disinfectants, Fungicides, and Chemical and Physical Sterilization. Second Edition, Lea & Febiger, Philadelphia, (1957).

T A B L E S

ERRATA

Page 11	"Stranis" should be "strains"
	Extend dividing line between "Plate" and "10^4" to separate "Replicate" and "Inoculum"
Page 14	Delete "O" following "f" in column headings
Page 41	Under both columns headed "Change, percent" the seventh and eighth entries should read "0.0" Under the second column headed "Before Treatment" delete all asterisks,*
Page 51	In the footnotes change "T^2/n" to "T^2/N"
Page 52	First entry under column "1" should read "86"
Page 64	In the footnotes change "T^2/n" to "T^2/N"
Page 72	Delete the "1:" portion of each of the column headings
Page 73	Delete the "1:" portion of each of the column headings
Page 74	Change "Gags" to Bags"

PHASE I

TABLE I

Colonies of B. Subtilis, var. niger*

Chemical	Replicate		Vehicle											
			a. 2% w/v Tide in Water				b. Acetone				c. Methanol			
			Dilution				Dilution				Dilution			
	Strip	Plate	$1:10^0$#	$1:10^1$	$1:10^2$	$1:10^3$	$1:10^0$#	$1:10^1$	$1:10^2$	$1:10^3$	$1:10^0$#	$1:10^1$	$1:10^2$	$1:10^3$
1. Ethylene Imine 5% v/v in vehicle	1	a	10	4	0	0	0	0	0	0	0	0	0	0
	1	b	8	0	0	0	0	0	0	0	0	0	0	0
	2	a	3	2	0	0	11	1	0	0	0	0	0	0
	2	b	7	2	0	0	13	0	0	0	0	0	0	0
	B.Cont.	a			TNC	TNC			TNC	TNC			TNC	TNC
	B.Cont.	b			TNC	TNC			TNC	TNC			TNC	TNC
2. Epichloro-hydrin 5% v/v in vehicle	1	a	1700	392	52	8	4000	868	16(3)	7	2	0	0	0(1)
	1	b	1900	348	58	9	1800	820	80(3)	13	4	0	0	0
	2	a	1500	162	57	4	1800	676	77	7	6	1	0	0
	2	b	1200	391	25	4	2000	601	64	9	8	0	0	0
	B.Cont.	a			TNC	TNC			TNC	TNC			TNC	TNC
	B.Cont.	b			TNC	TNC			TNC	TNC			TNC	TNC
3. Epibromo-hydrin 5% v/v in vehicle	1	a	1400	632	93	10	2500	581	60	8	1200	168	10	5
	1	b	1600	696	92	7	2000	576	69	13	1100	215	28	2
	2	a	1400	324	56	3	2000	577	64	6	282(3)	44	3	1(1)
	2	b	1500	352	36	5	2000	730	52	13	376	50	0	0
	B.Cont.	a			TNC	TNC			TNC	TNC			TNC	TNC
	B.Cont.	b			TNC	TNC			TNC	TNC			TNC	TNC
4. Formalde-hyde 5% w/v in vehicle	1	a	0	0	0	0	1	1	1	0	0	0(1)	0(1)	0
	1	b	0	0	0	0	2	0	0	0	0(1)	0	0	0
	2	a	10	2	0	0	9	0	0	0	0(1)	0	0	0
	2	b	3	0	0	0	2	1	0	0	0	0	0	0
	B.Cont.	a			TNC	TNC			TNC	TNC			TNC	TNC
	B.Cont.	b			TNC	TNC			TNC	TNC			TNC	TNC
5. β-Propio-lactone 5% v/v in vehicle	1	a	0	0	0	0	24	3	0	0	0	0	0	0
	1	b	0	0	0	0	17	1	0	0	0	0	0	0
	2	a	0	0	0	0	1	1	0	0	0	0	0	0
	2	b	0	0	0	0(1)	0	0	0	0	0	0	0	0
	B. Cont.	a			TNC	TNC			TNC	TNC			TNC	TNC
	B. Cont.	b			TNC	TNC			TNC	TNC			TNC	TNC

PHASE I

TABLE I (Cont.)

Colonies of B. Subtilis, var. niger*

Chemical	Replicate		Vehicle											
			d. Solvent M-17 Dilution				e. Solvent M-50 Dilution				f. Trichloroethylene Dilution			
	Strip	Plate	$1:10^0$#	$1:10^1$	$1:10^2$	$1:10^3$	$1:10^0$#	$1:10^1$	$1:10^2$	$1:10^3$	$1:10^0$#	$1:10^1$	$1:10^2$	$1:10^3$
1. Ethylene Imine 5% v/v in vehicle	1	a	0	0	0	0	2	0	0	0	0	0	0	0(2)
	1	b	0	0	0	0	1	0	0	0	0	0	0	0(1)
	2	a	0	0	0	0	0	0	0	0	0	0	0	0
	2	b	0	0	0	0	0	0	0(1)	0	0	0	0	0
	B.Cont.	a			TNC	TNC			TNC	TNC			TNC	TNC
	B.Cont.	b			TNC	TNC			TNC	TNC			TNC	TNC
2. Epichlorohydrin 5% v/v in vehicle	1	a	1700	678	61	8	>2000	808	132	11	1500	740	106	19
	1	b	2500	431	63	9	>2000	692	99	6	1300	680	132	12
	2	a	3500	493	86	18	6*	0*	0*	0*	900	142	10	8
	2	b	2000	543	79	8	6*	0(1)*	0*	0*	800	114	13	2
	B.Cont.	a			TNC	TNC			TNC	TNC			TNC	TNC
	B.Cont.	b			TNC	TNC			TNC	TNC			TNC	TNC
3. Epibromohydrin 5% v/v in vehicle	1	a	4000	614	88	38	1300	538		6	1900	652	85	6
	1	b	4000	493	102	14	1600	480	87	9	1700	508	70	9
	2	a	2000	356	62	4	1000	288	40	5	1300	488	50(1)	7(1)
	2	b	1600	408	56	0	1200	360	44	3	1100	396	46	5(5)
	B.Cont.	a			TNC	TNC			TNC	TNC			TNC	TNC
	B.Cont.	b			TNC	TNC			TNC	TNC			TNC	TNC
4. Formaldehyde 5% w/v in vehicle	1	a	526	46	8	1(1)	756	159	17	1	67	4	0	0
	1	b	472	47	11	1	1000	156	10	8	22	6	0	0(1)
	2	a	46	5	0	0	850	151	9	1	0	0	0	0
	2	b	35	8	0	0	1050	155	16	4	0	0	0	0(2)
	B.Cont.	a			TNC	TNC			TNC	TNC			TNC	TNC
	B.Cont.	b			TNC	TNC			TNC	TNC			TNC	TNC
5. B-Propiolactone 5% v/v in vehicle	1	a	0	0	0	0	696	116	13	1	776	97	16	1
	1	b	0	0	0	0	944	121	14	1	556	91	11	2
	2	a	0	0	0	0(1)	21	4	0	1(2)	29	4	2	0
	2	b	0	0	0	0	36	9	3(1)	0(6)	29	2	1	0(1)
	B.Cont.	a			TNC	TNC			TNC	TNC			TNC	TNC
	B.Cont.	b			TNC	TNC			TNC	TNC			TNC	TNC

TABLE I (Cont.)

* After seven days at 37° C ± 2° C on Trypticase soy agar/Hyland. The number of colonies of contaminating microorganisms is shown in parenthesis.

\# If all spores developed colonies when incubated on Trypticase soy agar/Hyland, the expected number of colonies developing would be 10^4, except for the bacteriostatis controls where 10^4 colonies would be expected for all dilutions.

* Specimen was dropped and was possibly scrubbed with the inoculum out of the water.

PHASE I

TABLE II

Summary of Table I

Total Number of Colonies of B. subtilis, var. niger for the Dilutions $1:10^1$, $1:10^2$, $1:10^3$

Chemical	Replicate		Vehicle					
	Strip	Plate	2% w/v Tide in Water	Acetone	Methanol	Solvent M-17	Solvent M-50	Trichloro-ethylene
Ethylene Imine	1	a	4	0	0	0	0	0
	1	b	0	1	0	0	0	0
	2	a	2	0	0	0	0	0
	2	b	2	0	0	0	0	0
Epichloro-hydrin	1	a	452	891	0	747	951	865
	1	b	415	913	0	503	797	824
	2	a	223	760	1	597	0	160
	2	b	420	674	0	630	0	129
Epibromo hydrin	1	a	735	649	183	740	544	743
	1	b	795	658	245	609	576	587
	2	a	388	647	48	422	333	545
	2	b	393	795	50	464	407	447
Formaldehyde	1	a	0	2	0	55	177	4
	1	b	0	0	0	59	174	6
	2	a	2	0	0	5	161	0
	2	b	0	1	0	8	175	0
β -Propio-lactone	1	a	0	3	0	0	130	114
	1	b	0	1	0	0	136	104
	2	a	0	1	0	0	5	6
	2	b	0	0	0	0	12	3

PHASE I

TABLE III

Analysis of Variance in Colonies of B. subtilis, var. niger

Factor	Level	n_*	T_*	$\Sigma T_*^2/n_*$	S_*	df	MS
i, plates	a	60	12,295				
	b	60	12,013	4,924,653	662	1	662
j, strips	1	60	15,392				
	2	60	8,916	5,273,478	349,487	1	349,487
k, chemicals	1	24	9				
	2	24	10,952				
	3	24	12,003				
	4	24	829				
	5	24	515	11,040,452	6,116,461	4	1,529,115
l, vehicles	a	20	3,831				
	b	20	5,996				
	c	20	527				
	d	20	4,839				
	e	20	4,578				
	f	20	4,537	5,793,234	869,243	5	173,849
kl, interaction between chemicals and vehicles	1a	4	8				
	1b	4	1				
	1c	4	0				
	1d	4	0				
	1e	4	0				
	1f	4	0				
	2a	4	1,510				
	2b	4	3,238				
	2c	4	1				
	2d	4	2,477				
	2e	4	1,748				
	2f	4	1,978				

PHASE I

TABLE III (Cont.)

Analysis of Variance in Colonies of B. subtilis, var. niger

Factor	Level	n_*	T_*	$\sum T^2_* / n_*$	S_*	df	MS
	3a	4	2,311				
	3b	4	2,749				
	3c	4	526				
	3d	4	2,235				
	3e	4	1,860				
	3f	4	2,322				
	4a	4	2				
	4b	4	3				
	4c	4	0				
	4d	4	127				
	4e	4	687				
	4f	4	10				
	5a	4	0				
	5b	4	5				
	5c	4	0				
	5d	4	0				
	5e	4	283				
	5f	4	227	13,377,279	1,467,576	29	506,061
Residuals			-	-	1,382,132	79	174,953
Total		120	24,308	4,923,991	10,185,561	119	-

$T = 24,308$
$N = 120$
$T^2/N = 4,923,991$
$\sum x^2 = 15,109,552$

PHASE I

TABLE IV

Analysis of Variance in Number in Colonies of B.subtilis, var. niger*

Factor	Level	n_*	T_*	$\Sigma T_*^2/n$	S_*	df	MS
i,plates	a	60	13,246				
	b	60	12,810	5,659,210	2,048	1	2,048
j,strips	1	60	15,392				
	2	60	10,664	5,843,909	186,747	1	186,747
k,chemical	1	24	9				
	2	24	12,698				
	3	24	12,003				
	4	24	829				
	5	24	515	12,760,990	7,103,828	4	1,775,957
l,vehicle	a	20	3,831				
	b	20	5,996				
	c	20	527				
	d	20	4,839				
	e	20	6,326				
	f	20	4,537	6,746,244	1,089,082	5	217,816
kl, inter-	1a	4	8				
action	1b	4	1				
between	1c	4	0				
chemicals	1d	4	0				
and	1e	4	0				
vehicles	1f	4	0				
	2a	4	1,510				
	2b	4	3,238				
	2c	4	1				
	2d	4	2,477				
	2e	4	3,496				
	2f	4	1,978				

PHASE I

TABLE IV (Cont.)

Analysis of Variance in Number in Colonies of B. subtilis, var. niger*

Factor	Level	n_*	T_*	$\Sigma T_*^2/n_*$	S_*	df	MS
	3a	4	2311				
	3b	4	2749				
	3c	4	526				
	3d	4	2235				
	3e	4	1860				
	3f	4	2322				
	4a	4	2				
	4b	4	3				
	4c	4	0				
	4d	4	127				
	4e	4	687				
	4f	4	10				
	5a	4	0				
	5b	4	5				
	5c	4	0				
	5d	4	0				
	5e	4	283				
	5f	4	227	15,668,900	1,818,828	29	627,182
Residuals		-	-	-	791,467	79	10,019
Total		120	26,056	5,657,626	10,992,000	119	-

T = 26,056
N = 120
T^2/N = 5,657,626
Σx = 16,649,162

* Based on the assumption that the second replicate results for 5% v/v Epichlorohydrin in Solvent M-50 were in error and should have been identical with those of the first replicate.

PHASE I

TABLE V

Spore Assays (Control 1)

Colonies of B. subtilis var. niger*

Date of Assay	Replicate		Dilution **					
	Suspension	Plate	$1:10^5$	$1:10^6$	$1:10^7$	$1:10^8$	$1:10^9$	$1:10^{10}$
6-8	2+	a	TNC	TNC	94	17		
		b	TNC	TCC	133	16		
6-9	1+	a	TNC	800	64	9		
		b	TNC	604	259	4		
7-3	1≠	a			100			
		b			91			
	2≠	a			89			
		b			72			
	3≠	a			63			
		b			84			
	4≠	a			93			
		b			76			
	5≠	a			110			
		b			99			
	6≠	a			142			
		b			216			
8-11	1≠	a		1004	115	12	3	1
		b		736	90	21	6	0
	2≠	a		860	70	10	0	0
		b		776	93	13	2	1

* After three days at 37°C on Trypticase soy agar/Hyland.

** Equivalent to having started with a suspension containing 10^9 spores/ml.

\+ From initial stock suspension of 10^9 spores/ml.

≠ From a dilution of the stock suspension prepared at 10^6 spores/ml on 6-30 after the stock suspension had partially frozen.

≠ From a dilution of the stock suspension prepared at 10^8 spores/ml. on 6-30.

From a dilution of the stock suspension prepared at 10^8 spores/ml. on 7-17.

PHASE I

TABLE VI

Effect of Heat Shock and Ultrasonics on Apparent Viability of Spores

Colonies of B. subtilis var. niger*

Specimen	Replicate	Dilution			
		$1:10^0$#	$1:10^1$	$1:10^2$	$1:10^3$
Control 1	1a	TNC	800	64	9
	1b	TNC	604	259	4
	2a	TNC	TNC	94≠	17≠
	2b	TNC	TCC	133≠	16≠
Ultrasonic	1a	TNC	600	170	400+
	1b	TNC	1200	69	6
Heat Shock	1a		870	55	22
	1b		700	60	4
Control 2	1a	TNC	515	64	12
	1b	TNC	1200	69	4
	2a	TNC	450	39	7
	2b	TNC	348	251	3

* After three days at 37°C on Trypticase soy agar / Hyland.

\# If all spores developed colonies when incubated on Trypticase soy agar, the expected number of colonies would be 10^4.

\+ Possibly not valid because of excess liquid remaining on the plate which permitted satellite colonies to develop.

≠ These had been plated on June 8; all others were plated on June 9.

PHASE I

TABLE VII

Ethylene Oxide Resistance** of Two Different Stranis of B. subtilis var. niger

Colonies of B. subtilis var. niger*

Spore Strain	Replicate		Inoculum		
	Bag	Plate	10^4	10^5	10^6
JPL	1	a	0(2)	0	0
		b	0(2)	0	0
	2	a	0	0	1
		b	0	0	2
BS4 of ATCC No. 9372	1	a	0	0(3)	14
		b	0	0(3)	18(1)
	2	a	0	0	27(3)
		b	0	0	25(3)

* After seven days at 37°C on Trypticase soy agar/Hyland.

** The spores were dried from distilled water on polyethylene film. The specimens were placed in either of two polyethylene bags where they were exposed to ethylene oxide by the standard process described elsewhere in this report, for three hours.

TABLE VIII

Infrared Spectra of Mixtures Compared with Calibration Spectra of Chemicals and Vehicles

Original Mixtures

	a	b	c	d	e	f
1		‡		‡	‡	-
2		-		-	-	-
3		+		-	-	+
4		+		+	+	+
5		+		+	-	+

In this table (++) indicates that some reaction occurred, (+) indicates possible reaction and (-) no apparent reaction. Criteria for the above evaluations were as follows: (1) (++) appearance of new peaks in the infrared spectrum accompanied by a corresponding disappearance or decrease in absorbance of one or more of the characteristic frequencies for the "chemicals", (2) (+) Increase or decrease of one or more frequency but with no corresponding opposite change, and (3) (-) no apparent change in relative absorbance.

Heated Mixtures

	a	b	c	d	e	f
1		-		-	+	-
2		-		-	-	-
3		-		-	-	-
4		-		+	-	-
5		-		-	-	-

(+) indicates continued change in the infrared spectrum upon heating and (-) indicates essentially no change in the spectrum upon heating.

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TABLE IX

Appearance of Chemical - Vehicle Combinations in Polyethylene containers when stored at room Temperature

Chemical	Vehicle						
	a	b	c	d	e	f	o
1				no liquid*	White ppt	no liquid white crust	Dissolved container and left white deposit
2							
3						brown spheres on liquid	
4							
5					white ppt		formed ppt in glass bottle
0						no liquid	

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TABLE IX (Cont.)

Appearance of Chemical - Vehicle Combinations in Polyethylene containers when stored at room Temperature
Heated Mixtures

Chemical	Vehicle						
	a	b	c	d	e	f	0
1				yellow crust in liquid	white crust in liquid	no liquid white crust	
2				no liquid white crust		white crust no liquid	
3				no liquid white crust		no liquid white crust brown deposit	
4				no liquid white crust			
5				no liquid pale pink crust		no liquid white crust	

In some cases the liquid may have all evaporated but in others, the analyst may have used the entire sample. No comments are shown when the bottle and its contents showed no change. The white precipitate appeared in the 5e bottle actually used to spray magnesium strips. All other bottles actually used to spray strips showed no change.

PHASE I

TABLE X

Effect of Aging and of Tide on the Sterilizing Effectiveness of 5% v/v B-Propiolactone in Vehicle during 90 Minutes contact in Petri dish

Colonies of B. subtilis, var. niger**

Vehicle	Replicate		Inoculum		
	Strip	Plate	10^6 (10^4)*	10^7 (10^5)*	10^8 (10^6)*
2% v/v Tide in Distilled Water, 14 days old.	1	a	TNC	TNC	TNC
	1	b	TNC	TNC	TNC
	2	a	TNC	TNC	TNC
	2	b	TNC	TNC	-
	3	a	TNC	TNC	TNC
	3	b	TNC	TNC	TNC
2% w/v Tide in Distilled Water, fresh solution	1	a	0	0	0
	1	b	0	0	0
Distilled Water	1	a	0	0	0
	1	b	0	0	0
	2	a	0	0	0
	2	b	0	0	0

** In one day on Trypticase soy agar/Hyland at 37°C.

* The maximum number of colonies expected, if all the spores in the inoculum developed colonies when placed on Trypticase soy agar.

PHASE I

TABLE XI

Relative Volatility of Several Chemical - Vehicle Combinations

Seconds to dry

Chemical / Vehicle	Ethylene Imine	Epichlorohydrin	Epibromohydrin	Formaldehyde	B-Propiolactone
2% w/v Tide in Water	>5 min	>5 min	>5 min	>5 min	>5 min
Acetone	75	44	143[1]	196[4]	216[3]
Methanol	128	71	161[8]	253[6]	171[1]
Solvent M-17	12	35	44	193[6]	135[5]
Solvent M-50	5	43	104	37	55[7]
Trichloroethylene	24	45	35	34	225[9]

1. B-Propiolactone in Methanol left a gummy deposit.
2. Epibromohydrin in Acetone left a small deposit.
3. B-Propiolactone in Acetone left a small deposit.
4. Formaldehyde in Acetone left deposit.
5. B-Propiolactone in M-17 left small deposit.
6. Formaldehyde in M-17 left deposit.
7. B-Propiolactone in M-50 left small deposit.
8. *While drying, small bubbles left on surface of Magnesium Alloy strip.
9. B-Propiolactone in Trichloroethylene left gummy deposit.

PHASE II

TABLE I

Colonies of B. subtilis, var. niger*

Subject	Replicate		Candidate Sterilant															
			5% v/v B-Propiolactone in Distilled Water				5% v/v B-Propiolactone in Solvent M-17				5% v/v Ethylene Imine in Trichloroethylene				5% w/v Formaldehyde in Methanol			
			Dilution			Date of Test	Dilution			Date of Test	Dilution			Date of Test	Dilution			Date of Test
	Specimen	Plate	$1:10^0$#	$1:10^1$	$1:10^2$		$1:10^0$#	$1:10^1$	$1:10^2$		$1:10^0$#	$1:10^1$	$1:10^2$		$1:10^0$#	$1:10^1$	$1:10^2$	
a	1	a	0	0	0	7-10	0	0	0	7-18	0	0	0	7-20	0	0	0	7-24
	1	b	0	0	0	7-10	0	0	0	7-18	0	0	0	7-20	0	0	0	7-24
	2	a	0	0	0	7-10	0(1)	0	0	7-18	0	0	0	7-20	0	0	0	7-24
	2	b	0	0	0	7-10	0	0	0	7-18	0	0	0	7-20	0	0	0	7-24
	3	a	0	0	0	7-10	0	0	0	7-18	0	0(1)	0	7-20	0	0	0	7-24
	3	b	0	0	0	7-10	0	0	0	7-18	0	0	0	7-20	0	0	0	7-24
	B.Cont.+	a	252	186		7-10	206	196		7-18	386	122		7-20	16	21		7-24
	B.Cont.+	b	205	168		7-10	187	176		7-18	377	376		7-20	17	12		7-24
	Cont.2	a	572	77	16	7-26												
	Cont.2	b	398	79	6	7-26												
e	1	a	0	0	0	7-24	0	0	0	7-10	0	0	0	7-17	0	0	0	7-24
	1	b	0	0	0	7-24	0	0	0	7-10	0	0	0	7-17	0	0	0	7-24
	2	a	0	0	0	7-24	0	0	0	7-10	0	0	0	7-17	0	0	0	7-24
	2	b	0	0	0	7-24	0	0	0	7-10	2	0	0	7-17	0	0	0	7-24
	3	a	0	0	0	7-24	0	0	0	7-10	0	0	0	7-17	0	0	0	7-24
	3	b	0	0	0	7-24	0	0	0	7-10	0	0	0	7-17	0	0	0	7-24
	B.Cont.+	a	0	134		7-24	169	100		7-10	149	136		7-17	2	22		7-24
	B.Cont.+	b	0	117		7-24	119	119		7-10	111	130(1)		7-17	2	20		7-24
	Cont.2	a	TNC	188	16	7-26												
	Cont.2	b	TNC	157	15	7-26												
f	1	a	0	0	0	7-24	0	0	0	7-24	0	0	0	7-10	0	0	0(8)	7-18
	1	b	0	0	0	7-24	0(1)	0	0	7-24	0	0	0	7-10	0	0	0	7-18
	2	a	0	0	0(2)	7-24	0	0(1)	0	7-24	0	0	0	7-10	0	0	0	7-18
	2	b	0	0	0	7-24	0(1)	0	0	7-24	0	0	0	7-10	0	0	0	7-18
	3	a	0	0	0	7-24	0	0	0	7-24	0	0	0	7-10	0	0	0	7-18
	3	b	0	0	0	7-24	0	0	0	7-24	0(1)	0	0	7-10	0	0	0	7-18
	B.Cont.+	a	10	34		7-24	234	676		7-24	64	35		7-10	147	132		7-18
	B.Cont.+	b	9	23		7-24	208	516(1)		7-24	89	36		7-10	107	130		7-18
	Cont.2	a	399	77	9	7-26												
	Cont.2	b	485	79	9	7-26												

PHASE II

TABLE I (Cont.)

Subject	Replicate		Candidate Sterilant															
			5% v/v B-Propiolactone in Distilled Water				5% v/v B-Propiolactone in Solvent M-17				5% v/v Ethylene Imine in Trichloroethylene				5% w/v Formaldehyde in Methanol			
	Specimen	Plate	Dilution			Date of Test	Dilution			Date of Test	Dilution			Date of Test	Dilution			Date of Test
			$1:10^0$#	$1:10^1$	$1:10^2$		$1:10^0$#	$1:10^1$	$1:10^2$		$1:10^0$#	$1:10^1$	$1:10^2$		$1:10^0$#	$1:10^1$	$1:10^2$	
g	1	a	0	0	0	7-24	2	0	0	7-18	0	0	0	7-20	0	0	0	7-10
	1	b	0	0	0	7-24	0	0	0	7-18	0	0	0	7-20	0	0	0	7-10
	2	a	0	0	0	7-24	0	0	0	7-18	0	0	0	7-20	0	0	0	7-10
	2	b	0	0	0	7-24	0	0	0	7-18	0	0	0	7-20	0	0(1)	0	7-10
	3	a	0	0	0	7-24	1	0	0	7-18	0	0	0	7-20	0	0	0	7-10
	3	b	0	0	0	7-24	0	0	0	7-18	0	0	0	7-20	0	0	0	7-10
	B.Cont.+	a	40	20(1)		7-24	105	287		7-18	391	70(1)		7-20	198	150		7-10
	B.Cont.+	b	41	17		7-24	115	121		7-18	452	59		7-20	245	114		7-10
	Cont.2	a	TNC	360	33	7-26												
	Cont.2	b	TNC	314	37	7-26												
h	1	a	0	0	0	7-13	0	0	0	7-24	0	0	0	7-24	0	0	0	7-18
	1	b	0	0	0	7-13	0	0	0	7-24	0	0	0	7-24	0	0(1)	0	7-18
	2	a	0	0	1	7-13	0	0	0	7-24	0	0	0	7-24	0	0	0	7-18
	2	b	0	0	0	7-13	0	0	0	7-24	0	0	0	7-24	0	0	0(2)	7-18
	3	a	0	0	0	7-13	0	0	0	7-24	0	0	0	7-24	0	0(1)	0	7-18
	3	b	2	0	0	7-13	0	0	0	7-24	0	0	0	7-24	0	0	0	7-18
	B.Cont.+	a	185	110		7-13	12	21		7-24	38	5		7-24	417	250		7-18
	B.Cont.+	b	156	117		7-13	29(1)	14		7-24	36	1		7-24	369	161		7-18
	Cont.2	a	137	16		8-2												
	Cont.2	b	131	30	22	8-2												
i	1	a	0	0	0	7-20	0	0	0	7-24	2	0	0	7-17	0	0	0	7-10
	1	b	0	0	0	7-20	0	0	0	7-24	1	+0(238)	0	7-17	0	0	0	7-10
	2	a	0	0(1)	0	7-20	0(1)	0	0	7-24	2	0	0	7-17	0	0	0	7-10
	2	b	0	0	0(1)	7-20	0(1)	0	0	7-24	0(1)	0	0	7-17	0	0	0	7-10
	3	a	0	0	0	7-20	0	0	0	7-24	1	0	0	7-17	0	0	0	7-10
	3	b	0	0	0	7-20	0	0	0	7-24	1	0	0	7-17	0	0	0	7-10
	B.Cont.+	a	5	0		7-20	110	21		7-24	98	56		7-17	6	134		7-10
	B.Cont.+	b	10	2		7-20	107	23		7-24	92	90		7-17	16	142		7-10
	Cont.2	a	195	41	4	7-26												
	Cont.2	b	245	23	4	7-26												

PHASE II

TABLE I (Cont.)

Subject	Replicate		Candidate Sterilant															
			5% v/v B-Propiolactone in Distilled Water				5% v/v B-Propiolactone in Solvent M-17				5% v/v Ethylene Imine in Trichloroethylene				5% w/v Formaldehyde in Methanol			
	Specimen	Plate	Dilution			Date of Test	Dilution			Date of Test	Dilution			Date of Test	Dilution			Date of Test
			$1:10^0$#	$1:10^1$	$1:10^2$		$1:10^0$#	$1:10^1$	$1:10^2$		$1:10^0$#	$1:10^1$	$1:10^2$		$1:10^0$#	$1:10^1$	$1:10^2$	
j	1	a	0(12)	0	0	7-18	0	0	0	7-20	0	0(1)	0	7-10	0	0	0	7-20
	1	b	0	0	0	7-18	0	0	0	7-20	0	0	0	7-10	0	0	0	7-20
	2	a	0	0	0	7-18	0	0	0	7-20	0	0	0	7-10	0(2)	0	1	7-20
	2	b	0	0	0	7-18	0	0	0	7-20	0	0	0	7-10	0	0	0	7-20
	3	a	0	0	0	7-18	0	0	0	7-20	0	0	0	7-10	0	0	0	7-20
	3	b	0	0(1)	0	7-18	0	0	0	7-20	0	0	0	7-10	0	0	0	7-20
	B.Cont.+	a	138	153		7-18	28	31		7-20	1/	76		7-10	51	176		7-20
	B.Cont.+	b	117	147		7-18	29	20		7-20	0/	123		7-10	51	186		7-20
	Cont.2	a	34	5	1	7-26												
	Cont.2	b	44	2	0	7-26												
k	1	a	0	0	0	7-18	32	4	1	7-20	5	2	0	7-24	0	0	0	7-11
	1	b	0	0	0	7-18	19	3	2	7-20	5	2	2	7-24	0	0	0	7-11
	2	a	0(1)	0	0	7-18	1	0	0	7-20	2	0	0	7-24	0	0	0	7-11
	2	b	0(1)	0(3)	0	7-18	0	0	0	7-20	2	0	0	7-24	0	0	0	7-11
	3	a	0	0	0	7-18	0	0	0	7-20	0	0	0	7-24	0	0	0	7-11
	3	b	0	0	0	7-18	0	0	0	7-20	0	0	0	7-24	0	0	0	7-11
	B.Cont.+	a	93	115		7-18	75	217		7-20	40	5		7-24	278	2		7-11
	B.Cont.+	b	109	175(1)		7-18	61	146		7-20	58	4		7-24	278	4		7-11
	Cont.2	a																
	Cont.2	b																
k'	1	a	0	0	0	8-1	0	0	0	8-1	0	0	0	8-1	0	0	0	8-1
	1	b	0	0	0	8-1	0	0	0	8-1	0	0	0	8-1	0	0	0	8-1
	2	a	0	0	0	8-1	0	0(2)	0	8-1	0	0	0	8-1	0	0	0	8-1
	2	b	0	0	0	8-1	0	0(1)	0	8-1	0	0	0	8-1	0	0	0	8-1
	3	a	0	0	0	8-1	0	0	0	8-1	0	0	0	8-1	0	0	0(4)	8-1
	3	b	0	0	0	8-1	0	0	0	8-1	0	0	0	8-1	0	0	0	8-1
	B.Cont.	a	TNC#	TNC		8-1	TNC#	TNC		8-1	TNC#	777		8-1	TNC#	416		8-1
	B.Cont.	b	TNC#	TNC		8-1	TNC#	TNC		8-1	TNC#	800		8-1	TNC#	520		8-1
	Cont.2	a	TNC	476	65	8-1												
	Cont.2	b	TNC	284	55	8-1												

PHASE II
TABLE I (Cont.)

Subject	Replicate		Candidate Sterilant: 5% v/v B-Propiolactone in Distilled Water				5% v/v B-Propiolactone in Solvent M-17				5% v/v Ethylene Imine in Trichloroethylene				5% w/v Formaldehyde in Methanol			
			Dilution			Date of Test	Dilution			Date of Test	Dilution			Date of Test	Dilution			Date of Test
	Specimen	Plate	$1:10^0$ #	$1:10^1$	$1:10^2$		$1:10^0$ #	$1:10^1$	$1:10^2$		$1:10^0$ #	$1:10^1$	$1:10^2$		$1:10^0$ #	$1:10^1$	$1:10^2$	
l	1	a	0	0	0	7-10	8	0	0	7-18	2	0	0	7-24	0	0	0	7-20
	1	b	0	0	0	7-10	7	0	0	7-18	2	2	0	7-24	0	0	0	7-20
	2	a	0	0	0	7-10	25	4	0	7-18	0	0	0	7-24	0	0	0	7-20
	2	b	2	0	0	7-10	30	1	0	7-18	0	0	0	7-24	0	0	0	7-20
	3	a	0	0	0	7-10	1	2	0	7-18	0	0	0	7-24	0	0	0	7-20
	3	b	0	0	0	7-10	3	1	0	7-18	0	0	0	7-24	0	0	0	7-20
	B.Cont.+	a	189	130		7-10	196	140		7-18	0(1)≠	12		7-24	105	225		7-20
	B.Cont.+	b	183	93		7-10	176	125		7-18	0 ≠	9		7-24	124	366		7-20
	Cont.2	a	TNC	636	112	7-26												
	Cont.2	b	TNC	876	113	7-26												
m	1	a	0	0	0(1)	7-20	0	0	0	7-11	0	0	0	7-24	0	0	0(1)	7-18
	1	b	0	0	0	7-20	0	0	0	7-11	0	0	0	7-24	0	0	0	7-18
	2	a	0	0(1)	0	7-20	0	0	0	7-11	0	0(1)	0(1)	7-24	0	0	0	7-18
	2	b	0	0	0	7-20	0	0	0	7-11	0	0	0	7-24	0	0	0	7-18
	3	a	0	0	0	7-20	0	0	0	7-11	0	0	0	7-24	0	0	0	7-18
	3	b	0	0	0	7-20	0	0	0	7-11	0	0	0	7-24	0	0	0	7-18
	B.Cont.+	a	221	96		7-20	151	157		7-11	52	11		7-24	84	6		7-18
	B.Cont.+	b	135	150		7-20	112	159		7-11	45	12		7-24	106	11		7-18
	Cont.2	a	TNC	972	154	7-26												
	Cont.2	b	TNC	636	198	7-26												
n	1	a	0	0	0	7-20	0(1)	0(1)	0	7-18	0	0	0	7-10	0	0	0	7-24
	1	b	0	0	0	7-20	0	0	0	7-18	0	0	0	7-10	0	0	0	7-24
	2	a	0	0	0	7-20	0(2)	0	0	7-18	0	0	0	7-10	0	0	0	7-24
	2	b	0	0	0	7-20	0	0	0	7-18	0	0	0	7-10	0	0	0	7-24
	3	a	0	0	0	7-20	4	0	0	7-18	0	0	0	7-10	0	0	0	7-24
	3	b	0	0	0	7-20	0	0	0	7-18	0	0	0	7-10	0	0	0	7-24
	B.Cont.+	a	138	195		7-20	172	124		7-18	144	62		7-10	12	18		7-24
	B.Cont.+	b	135	134		7-20	145	135		7-18	146	99		7-10	11	17		7-24
	Cont.2	a	TNC	338	54	7-26												
	Cont.2	b	TNC	392	63(13	7-26												

PHASE II

TABLE I (Cont.)

Subject	Replicate		Candidate Sterilant															
			5% v/v B-Propiolactone in Distilled Water				5% v/v B-Propiolactone in Solvent M-17				5% v/v Ethylene Imine in Trichloroethylene				5% w/v Formaldehyde in Methanol			
			Dilution			Date of Test	Dilution			Date of Test	Dilution			Date of Test	Dilution			Date of Test
	Specimen	Plate	$1:10^0$#	$1:10^1$	$1:10^2$		$1:10^0$#	$1:10^1$	$1:10^2$		$1:10^0$#	$1:10^1$	$1:10^2$		$1:10^0$#	$1:10^1$	$1:10^2$	
o	1	a	0	0	0	7-18	0	0	0	7-13	0(1)	0	0	7-20	0	0	0	7-24
	1	b	0	0	0	7-18	0	0	0	7-13	0	0	0	7-20	0(3)	0	0	7-24
	2	a	0	0	0(1)	7-18	0	0	0	7-13	0	0	0	7-20	0	0	0	7-24
	2	b	0(1)	0	0	7-18	0	0	0	7-13	0	0	0	7-20	0	0	0	7-24
	3	a	0	0	0	7-18	0	0	0	7-13	0	0	0	7-20	0	0	0	7-24
	3	b	0	0	0	7-18	0	0	0	7-13	0	0	0	7-20	0	0	0	7-24
	B.Cont.+	a	106	203		7-18	153	183		7-13	71	64		7-20	12	12		7-24
	B.Cont.+	b	100	177		7-18	169	132		7-13	70	88		7-20	12	9		7-24
	Cont.2	a	TNC	652	102	7-26												
	Cont.2	b	TNC	432	75	7-26												
o'	1	a	0	0	0(1)	7-18	0	0	0	7-12	0(1)	0	0(2)	7-20	0	0	0	7-24
	1	b	0	0	0	7-18	0	0	0	7-12	0	0	0	7-20	0	0	0	7-24
	2	a	0	0(1)	0	7-18	0	0	0	7-12	0	0	0	7-20	0	0	0	7-24
	2	b	0	0	0	7-18	0	0	0	7-12	0	0(1)	0(2)	7-20	0	0	0	7-24
	3	a	0	0	0	7-18	0	0	0	7-12	0	0	0	7-20	0	0	0	7-24
	3	b	0(1)	0	0	7-18	0	0	1	7-12	0	0	0	7-20	0	0	0	7-24
	B.Cont.+	a	142	130		7-18	236	107		7-12	165	26		7-20	14	37		7-24
	B.Cont.+	b	128	95		7-18	172	99		7-12	172	15		7-20	20	20		7-24
	Cont.2	a	TNC	232	35	7-26												
	Cont.2	b	TNC	224	38	7-26												
p	1	a	0	0	0	7-11	0	0	0	7-20	0	0	0	7-17	0	0	0	7-24
	1	b	0	0	0	7-11	0	0	0	7-20	0	0	0	7-17	0	0(1)	0	7-24
	2	a	21	6	0	7-11	3	0	0	7-20	0	0	0	7-17	0	0	0	7-24
	2	b	15	2	2	7-11	0	0	0	7-20	0	0	0	7-17	0	0	0	7-24
	3	a	51(1)	6	0	7-11	0	0	0	7-20	0	0	0	7-17	0	0	0	7-24
	3	b	49	8	2	7-11	1	0	0	7-20	0	0	0	7-17	0	0	0	7-24
	B.Cont.+	a	211	188		7-11	60	20		7-20	118	112		7-17	15	21		7-24
	B.Cont.+	b	190	132		7-11	82	27		7-20	125	113		7-17	10	24		7-24
	Cont.2	a	TNC	391	43	7-26												
	Cont.2	b	TNC	402	56	7-26												

PHASE II

TABLE I (Cont.)

Subject	Replicate		Candidate Sterilant				
			5% v/v B-Propiolactone in Distilled Water				
	Specimen	Plate	Dilution			Date of Test	
			$1:10^0$#	$1:10^1$	$1:10^4$		
p'	1	a	0	0	0	8-1	
	1	b	0	0	0	8-1	
	2	a	0	0(1)	0	8-1	
	2	b	0	0(1)	0	8-1	
	3	a	0(99)	0(1)	0	8-1	
	3	b	0(106)	0(13)	0	8-1	
	B.Cont.	a	TNC#	TNC#		8-1	
	B.Cont.	b	TNC#	TNC#		8-1	
	Cont.2	a					
	Cont.2	b					

* After 7 days at 37° C. on Trypticase soy agar/Hyland.

\+ If all spores developed colonies when incubated on Trypticase soy agar, the expected number of colonies developing would be 10^2.

\# If all spores developed colonies when incubated on Trypticase soy agar, the expected number of colonies developing would be 10^4.

♠ A defective plate of some sort, as it was heavily contaminated around rim.

≠ These plates may not have been inoculated.

≠ Inoculating needle fell into jar. Probably too little inoculum.

PHASE II

TABLE II

Summary of Table I

Total Number of Colonies of B. subtilis, var. niger for All Dilutions, Plates, Specimens

Subject	Sterilants															
	A				B				C				D			
	Batch				Batch				Batch				Batch			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
a	0	-	-	-	-	0	-	-	-	-	0	-	-	-	-	0
e	-	-	-	0	0	-	-	-	-	2	-	-	-	-	0	-
f	-	-	-	0	-	-	0	-	0	-	-	-	-	0	-	-
g	-	-	-	0	-	3	-	-	-	-	0	-	0	-	-	-
h	3	-	-	-	-	-	-	0	-	-	0	-	-	0	-	-
i	-	-	1	-	-	-	-	0	-	7	-	-	0	-	-	-
j	-	0	-	-	-	-	-	0	0	-	-	-	-	-	1	-
k	-	1	-	-	-	-	62	-	-	-	-	20	0	-	-	-
k'*	(0)	-	-	-	(0)	-	-	-	(0)	-	-	-	(0)	-	-	-
l	2	-	-	-	-	82	-	-	-	-	-	6	-	-	0	-
m	-	-	0	-	0	-	-	-	-	-	-	0	-	0	-	-
n	-	-	0	-	-	4	-	-	0	-	-	-	-	-	-	0
o	-	0	-	-	0	-	-	-	-	-	0	-	-	-	-	0
o'	-	0	-	-	1	-	-	-	-	-	0	-	-	-	-	0
p	0+	-	-	-	-	-	4	-	-	0	-	-	-	-	-	0

PHASE II

TABLE II (Cont.)

Total Number of Colonies of B. subtilis, var. niger for All Dilutions, Plates, Specimens

* k'was exposed to the sterilants and tested for sterility by itself and therefore has no batch number.

\+ On the first test, using old B-propiolactone, 162 colonies of B. subtilis, var. niger appeared on the plates.

PHASE II

TABLE III

Analysis of Variance in Number of Colonies of B. subtilis, var. niger.

Factor	Level	n_*	T_*	$\sum T_*^2/n$	S_*	df	MS
Subject	a	4	0				
	e	4	2				
	f	4	0				
	g	4	3				
	h	4	3				
	i	4	8				
	j	4	1				
	k	4	83				
	l	4	90				
	m	4	0				
	n	4	4				
	o	4	0				
	o'	4	1				
	p	4	4	3777.25	3070.09	13	236.16
Sterilant	A	14	7				
	B	14	156				
	C	14	35				
	D	14	1	1829.36	1122.20	3	374.07
Batch	I	14	6				
	II	14	99				
	III	14	68				
	IV	14	26	1081.21	374.05	3	124.68
Residuals				-	5841.51	36	162.26
Total		56	199	707.16	10,407.84	55	-

$T = 199$
$N = 56$
$T^2/N = 707.16$
$\Sigma x^2 = 11,115.00$

PHASE II

TABLE IV

Summary of Table I

. Total Number of Colonies of Contaminants for all dilutions, plates, and specimens

Subject	Sterilant															
	A				B				C				D			
	Batch				Batch				Batch				Batch			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
a	o	-	-	-	-	1	-	-	-	-	1	-	-	-	-	o
e	-	-	-	o	o	-	-	-	-	o	-	-	-	-	o	-
f	-	-	-	2	-	-	3	-	1	-	-	-	-	o	-	-
g	-	-	-	o	-	o	-	-	-	-	o	-	1	-	-	-
h	o	-	-	-	-	-	-	o	-	-	o	-	-	4	-	-
i	-	-	2	-	-	-	-	2	-	1≠	-	-	o	-	-	-
j	-	13	-	-	-	-	-	o	1	-	-	-	-	-	2	-
k	-	5	-	-	-	-	o	-	-	-	-	o	o	-	-	-
k' *	(o)	-	-	-	(3)	-	-	-	(o)	-	-	-	(4)	-	-	-
l	o	-	-	-	-	o	-	-	-	-	-	o	-	-	o	-
m	-	-	2	-	o	-	-	-	-	-	-	1	-	1	-	-
n	-	-	o	-	-	4	-	-	o	-	-	-	-	-	-	o
o	-	2	-	-	o	-	-	-	-	-	1	-	-	-	-	3
o'	-	4	-	-	o	-	-	-	-	-	6	-	-	-	-	o
p	1+	-	-	-	-	-	o	-	-	o	-	-	-	-	-	1

PHASE II

TABLE IV (Cont.)

Summary of Table I

Total Number of Colonies of Contaminants for all dilutions, plates, and specimens

*k" was exposed to the sterilant and tested for sterility by itself. It therefore, has no batch number and is not included in the analysis of variance to follow.

+ On the second test, 221 contaminant colonies appeared.

≠ The plate was defective - 238 colonies of colonies appeared. The number shown in the table is the average number observed for all plates and is used so that the analysis of variance is not unduly biased by the defective plate.

PHASE II

TABLE V

Analysis of Variance in the Number of Contaminants

Factor	Level	n_*	T_*	$\Sigma T_*^2/n_*$	S_*	df	MS
Subject	a	4	2				
	e	4	0				
	f	4	6				
	g	4	1				
	h	4	4				
	i	4	5				
	j	4	16				
	k	4	5				
	l	4	0				
	m	4	4				
	n	4	4				
	o	4	6				
	o'	4	10				
	p	4	2	133.36	58.30	13	4.48
Sterilant	A	14	31				
	B	14	10				
	C	14	12				
	D	14	12	96.36	20.91	3	6.97
Batch	I	14	4				
	II	14	35				
	III	14	17				
	IV	14	9	115.07	36.62	3	13.21
Residuals +		-	-	-	136.72	36	3.80
Total		56	65	75.45	255.55	55	-

T = 65
N_* = 56
T^2/N = 75.45
Σx^2 = 331

\+ The residual variance includes that due to variations between plates and between specimens.

PHASE II — Summary of Table I — TABLE VI

Colonies of B. subtilis var. niger Appearing on Bacteriostasis Control Plates

Subject	Plate	Dilution	Sterilant A Batch I	A Batch II	A Batch III	A Batch IV	B Batch I	B Batch II	B Batch III	B Batch IV	C Batch I	C Batch II	C Batch III	C Batch IV	D Batch I	D Batch II	D Batch III	D Batch IV
a	a	$1:10^0$	252				206				386				16			
		$1:10^1$	186				196				122				21			
	b	$1:10^0$	205				187				377				17			
		$1:10^1$	168				176				376				12			
e	a	$1:10^0$	0				169				149				2			
		$1:10^1$	134				100				136				22			
	b	$1:10^0$	0				119				111				2			
		$1:10^1$	117				119				130				20			
f	a	$1:10^0$	10				234				64				147			
		$1:10^1$	34				676				35				132			
	b	$1:10^0$	9				208				89				107			
		$1:10^1$	23				516				36				130			
g	a	$1:10^0$	40				105				391				198			
		$1:10^1$	20				287				70				150			
	b	$1:10^0$	41				115				452				245			
		$1:10^1$	17				121				59				114			

PHASE II — Summary of Table I — TABLE VI (Cont.)

Colonies of B. subtilis, var. niger Appearing on Bacteriostasis Control Plates

Subject	Plate	Dilution	Sterilant											
			A				B				C			D
			Batch				Batch				Batch			Batch
			I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
h	a	$1:10^0$	185						12			38				417		
		$1:10^1$	110						21			5				250		
	b	$1:10^0$	156						29			36				369		
		$1:10^1$	117						14			1				161		
i	a	$1:10^0$		5					110			98				6		
		$1:10^1$		0					21			56				134		
	b	$1:10^0$		10					107			92				16		
		$1:10^1$		2					23			90				142		
j	a	$1:10^0$		138					28			I				51		
		$1:10^1$		153					31			0				176		
	b	$1:10^0$		117					29			76				51		
		$1:10^1$		147					20			123				186		
k	a	$1:10^0$		93					75			40				278		
		$1:10^1$		115					217			5				2		
	b	$1:10^0$		109					61			58				278		
		$1:10^1$		175					146			4				4		

PHASE II — Summary of Table I — TABLE VI (Cont.)

Colonies of B. subtilis, var. niger Appearing on Bacteriostasis Control Plates

Subject	Plate	Dilution	Sterilant A Batch I	A Batch II	A Batch III	A Batch IV	B Batch I	B Batch II	B Batch III	B Batch IV	C Batch I	C Batch II	C Batch III	C Batch IV	D Batch I	D Batch II	D Batch III	D Batch IV
k'	a	$1:10^0$	TNC				TNC				TNC				TNC			
		$1:10^1$	TNC				TNC				777				416			
	b	$1:10^0$	TNC				TNC				TNC				TNC			
		$1:10^1$	TNC				TNC				800				520			
l	a	$1:10^0$	189				196				0				105			
		$1:10^1$	130				140				12				225			
	b	$1:10^0$	183				176				0				124			
		$1:10^1$	93				125				9				366			
m	a	$1:10^0$		221			151				52				84			
		$1:10^1$		96			157				11				6			
	b	$1:10^0$		135			112				45				106			
		$1:10^1$		150			159				12				11			
n	a	$1:10^0$		138			172				144					12		
		$1:10^1$		195			124				62					18		
	b	$1:10^0$		135			145				146					11		
		$1:10^1$		134			135				99					17		

PHASE II

TABLE VI (Cont.)

Summary of Table I

Colonies of B. subtilis, var. niger Appearing on Bacteriostasis Control Plates

Subject	Plate	Dilution	Sterilant											
			A				B				C		D	
			Batch				Batch				Batch		Batch	
			I	II	III	IV	I	II	III	IV	I	II	III	IV
o	a	$1:10^0$	106				153				71			12
		$1:10^1$	203				183				64			12
	b	$1:10^0$	100				169				70			12
		$1:10^1$	177				132				88			9
o'	a	$1:10^0$	142				236				165			14
		$1:10^1$	130				107				26			37
	b	$1:10^0$	128				172				172			20
		$1:10^1$	95				99				15			20
p	a	$1:10^0$	211				60				118			15
		$1:10^1$	188				20				112			21
	b	$1:10^0$	190				82				125			10
		$1:10^1$	132				27				113			24

k' really has no batch numbers. This subject was tested after the testing of all other subjects was completed. The expected number of colonies of B. subtilis, var. niger appearing on the bacteriostasis control plates for this subject was one-hundred fold greater than that for the other subjects.

PHASE II - TABLE VII

Analysis of Variance in Number of Colonies on Bacteriostasis Controls

Factor	Level	n_*	T_*	$\sum T_*^2/n_*$	S_*	df	MS
Subject	a	16	2,903				
	e	16	1,330				
	f	16	2,450				
	g	16	2,425				
	h	16	1,921				
	i	16	912				
	j	16	1,327				
	k	16	1,660				
	l	16	2,073				
	m	16	1,508				
	n	16	1,687				
	o	16	1,561				
	o'	16	1,578				
	p	16	1,448	2,972,426	230,477	13	17,729
Plate	a	112	12,607				
	b	112	12,176	2,742,780	829	1	829
Dilution	0	112	13,157				
	1	112	11,626	2,752,415	10,466	1	10,466
Sterilant	A	56	6,489				
	B	56	7,710				
	C	56	5,437				
	D	56	5,147	2,814,353	72,405	3	24,135
Batch	I	56	7,474				
	II	56	7,984				
	III	56	7,857				
	IV	56	1,468	3,276,650	534,701	3	178,234
Residuals		—	—	—	1,427,635	202	7,068
Total		224	24,783	2,741,949	2,276,516	223	—

N_2 = 224
T_*^2/N = 2,741,949
$\sum x^2$ = 5,018,465
T = 24,783

PHASE II

TABLE VIII

Ranking of Subjects By Percent

Spore Recovery From "Control 2" Specimens

Subject	Percent Recovery
m	64 to 97
l	64 to 88
o	43 to 65
k'	28 to 47
p	39 to 40
n	34 to 39
g	31 to 36
o	22 to 23
e	16 to 19
a	8
f	7 to 8
i	2 to 4
h	1.6 to 3
j	0.2 to 0.5

TABLE IX

PHASE II

Appearance of Subject After Treatment

Subject	Replicate	Sterilant: 5% v/v B-Propiolactone in Distilled Water	Sterilant: 5% v/v B-Propiolactone in Solvent M-17
a	1	Discolorations on surface.	Normal
	2	Glossy spots.	Normal
b	1	Normal	Normal
	2	Normal	Normal
c	1	Still wet (browned) after 4 days. When	Some paint stuck to plate and
	2	blotted paint came off at wet spots.	came off specimen around edges.
e	1	Small brown spots.	Flattened out. Specimen was
	2	Normal	curled before treatment.
f	1	Normal	Normal
	2	Normal	Normal
g	1	Normal	Light film on shiny surface.
	2	Normal	Epoxy attacked.
h	1	Residue was oily fluid. It was dried	Residue was greasy.
	2	over Drierite for 48 hours.	
i	1	Normal	Dirty
	2	Normal	Dirty
j	1	Small deposit. Shiny	Glossy film on surface.
	2	surface slightly dull.	
k	1	Corroded	Surface dulled. Deposits.
	2	Corroded	Mating surfaces adhered.
k'	1	Discolored. Deposits looking like dried	Normal
	2	raw egg yolk. Brown liquid in bag.	Normal
l	1	Corroded outside and on flat mating	Surface dulled.
	2	surface. Threads clean.	Surface dulled.
m	1	Subject wet. Glossy spots on surface	Gummy deposit on occluded
	2	Brown spots.	surfaces (one only).
n	1	Rubber and tube wet.	Normal
	2		Normal
o	1	Normal	Gummy deposit on all surfaces.
	2	Normal	
o'	1	Housing spotted. Yellow	Gummy deposit on all surfaces.
	2	deposits wet insulation.	
p	1	Corroded except for nut and threads	Light film on surfaces.
	2	covered by nut.	
q	1	Slightly blistered. Still wet after	Normal except where lying on solvent
	2	24 hours.	covered glass surface, tacky and wrinkled there.

TABLE IX (Cont.)

PHASE II

Appearance of Subject After Treatment

Subject	Replicate	Sterilant: 5% v/v Ethylene Imine in Trichloroethylene	Sterilant: 5% w/v Formaldehyde in Methanol
a	1	Glossy spots.	Normal
	2	Normal	Normal
b	1	Paint puckered near edge of specimen	Some paint left on glass dish.
	2	Paint left on dish.	
c	1	Slightly tacky on shiny spots where	Normal
	2	sterilant dried last.	Normal
e	1	Normal	Normal
	2	Normal	Normal
f	1	Normal	Normal
	2	Normal	Normal
g	1	Normal	Normal
	2	Surface film.	Normal
h	1	The residue was greasy.	Residue was hard white deposit
	2		with formaldehyde odor.
i	1	Normal	Normal
	2	Normal	Normal
j	1	Light yellow-brown deposits on surface.	Normal
	2		Surface film.
k	1	White deposits.	Heavy deposit of paraformaldehyde
	2		on all occluded surfaces.
k'	1	Normal	A few white deposits.
	2	Slightly attacked on finish.	
l	1	Filmy deposit. Mating surfaces shiny.	Surface film and deposits on
	2		occluded surfaces.
m	1	Normal	Very small deposits and pits.
	2	Normal	Normal
n	1	Small amount of dark substance on	Deposits on threads of screw.
	2	threads under nut.	
o	1	Normal	Normal
	2	Normal	Normal
o'	1	Oily	Wet around pins. White film.
	2	Oily	
p	1	Surface dulled. Slight pitting. Corroded.	Exterior surfaces wet. White
	2	White deposits.	crystalline deposits.
q	1	Stuck to dish where exposure to solvent	Wet. Coating dissolved and
	2	was greatest.	recongealed.

PHASE II

Relative Change in Weight of Subjects Exposed to Sterilants

TABLE X

Subject	Replicate	5% v/v β-Propiolactone in Distilled Water			5% v/v β-Propiolactone in Solvent M-17			5% v/v Ethylene Imine in Trichloroethylene			5% w/v Formaldehyde in Methanol		
		Weight, Grams		Weight	Weight, Grams		Weight	Weight, Grams		Weight	Weight, Grams		Weight
		Before Treat.	After Treat.	Change Percent	Before Treat.	After Treat.	Change Percent	Before Treat.	After Treat.	Change Percent	Before Treat.	After Treat.	Change Percent
a	1	1.7154	1.7167	0.0758	1.7577	1.7589	0.0683	1.7588	1.7591	0.0171	1.7560	1.7558	-0.0114
	2	1.7583	1.7634	0.2901	1.7211	1.7219	0.0465	1.7473	1.7475	0.0114	1.7508	1.7505	-0.0171
b	1	11.2491	11.2515	0.0213	11.3722	11.3720	-0.0018	11.4117	11.4109	-0.0070	11.3295	11.3291	-0.0035
	2	11.2829	11.2855	0.0230	11.3897	11.3890	-0.0061	11.3678	11.3670	-0.0070	11.3504	11.3500	-0.0035
c	1	13.4786	13.4865	0.0586	11.4481	11.4506	0.0218	14.2882	14.2940	0.0406	11.6655	11.6691	0.0309
	2	14.3050	14.3130	0.0559	11.7667	11.7706	0.0331	11.3746	11.9810	0.0534	11.6264	11.6330	0.0568
e	1	1.3128	1.3133	0.0381	1.2804	1.2804	0.0000	0.9907	0.9908	0.0101	0.9860	0.9860	0.0000
	2	1.2401	1.2402	0.0081	1.2936	1.2935	-0.0077	0.8693	0.8694	0.0115	0.8991	0.8990	-0.0111
f	1	5.6909	5.7181	0.4780	5.4078	5.4430	0.6509	6.3401	6,3973	0.9022	6.7261	6.7591	0.4906
	2	5.2898	5.3171	0.5161	6.1157	6.1616	0.7505	5.0283	5.0926	1.2788	6.4044	6.4355	0.4856
g	1	57.3241	57.3421	0.0314	57.3520	57.3930	0.0715	57.1799	57.1875	0.0133	57.6538	57.6746	0.0361
	2	57.5481	57.5624	0.0248	57.2400	57.2996	0.1041	57.1088	57.1183	0.0166	57.5652	57.5855	0.0353
i	1	1.5556	1.5561	0.0321	1.5965	1.5953	-0.0752	1.5251	1.5242	-0.0590	1.6155	1.6152	-0.0185
	2	1.5965	1.6453	3.0577	1.6498	1.6477	-0.1273	1.5503	1.5493	-0.0645	1.5704	1.5700	-0.0254
j	1	13.3294	13.3357	0.0473	13.5666	13.6158	0.3627	13.8045	13.8763	0.5201	12.9849	12.9876	0.0208
	2	13.1120	13.1181	0.0465	13.9438	13.9751	0.2245	13.7259	13.8025	0.5581	13.2643	13.2671	0.0211
q	1	3.3276	3.3193	-0.2494	3.3158	3.3183	0.0754	3.3726	3.3522	-0.6049	3.4682	3.4575	-0.3085
	2	3.3546	3.3465	-0.2385	3.4600	3.4613	0.0376	3.3981	3.3783	-0.5827	3.3072	3.2984	-0.2661

PHASE II
TABLE XI

Analysis of Variance of Relative Change in Weight

Factor	Level	n_*	T_*	T_*^2/n_*	S_*	df	MS	MS+
Subject	a	8	0.4807					
	b	8	0.0154					
	c	8	0.3511					
	e	8	0.0490					
	f	8	5.5527					
	g	8	0.3331					
	i	8	2.7199					
	j	8	1.8011					
	q	8	-2.1371	5.8137	4.6468	8	0.5809	0.5471
Replicates	1	36	2.7758					
	2	36	6.3901	1.3483	0.1814	1	0.1814	0.0048
Sterilants	A	18	4.3169					
	B	18	2.2286					
	C	18	2.1081					
	D	18	0.5121	1.5728	0.4059	3	0.1353	0.0355
Residuals		-	-	-	9.2885	59	0.1574	0.0226
Total		72	9.1659	1.1669	14.5266	71	-	-

$T = 9.1659$

$N = 72$

$T^2/N = 1.1669$

$x^2 = 15.6935$

+ Calculated on the basis of using a value of 0.0321 instead of the value of 3.0577 for replicate 2 of subject i exposed to sterilant A. The latter value appears to be an outlier. The conclusion to be drawn from the analysis of variance are not altered significantly by rejecting this outlier.

PHASE II

TABLE XII

Change in Dimension Resulting from Exposure to Sterilants

Subject	Replicate	Sterilant											
		5% v/v B-Propiolactone in Distilled Water			5% v/v B-Propiolactone in Solvent M-17			5% v/v Ethylene Imine in Trichloroethylene			5% w/v Formaldehyde in Methanol		
		Dimension, inches			Dimension, inches			Dimension, inches			Dimension, inches		
		Before Treatment	After Treatment	change, percent	Before Treatment	After Treatment	change, percent	Before Treatment	After Treatment	change, percent	Before Treatment	After Treatment	change, percent
e	1	0.0100	0.0103	3.00	0.0100	0.0100	0.00	0.0100	0.0100	0.00	0.0101	0.0103	3.00
	2	0.0100	0.0103	3.00	0.0100	0.0100	0.00	0.0100	0.0100	0.00	0.0101	0.0102	2.00
1	1	0.2369	0.2387	0.75	0.2385	0.2439	2.26	0.2357	0.2393	1.53	0.2295	0.2328	1.44
	2	0.2449	0.2476	1.10	0.2361	0.2412	2.16	0.2423	0.2458	1.44	0.2285	0.2328	1.88
1	1	0.2055	0.2053	0.097	0.2026	0.2033	0.35	0.2038	0.2043	0.245	0.1956	0.1956	0.00
	2	0.2035	0.2005	1.47	0.2042	0.2050	0.39	0.2010	0.2015	0.248	0.2000	0.2001	0.05

PHASE II

TABLE XIII

Analysis of Variance in Change in Dimension

Factor	Level	n_*	T_*	$\Sigma T_*^2/n_*$	S_*	df	MS
Subjects	e	16	0.1613				
	i	16	3.8145				
	j	16	3.2318	1.5638	0.4816	2	0.2408
Replicates	1	24	3.5920				
	2	24	3.6156	1.0822	0.0	1	0.0
Sterilants	A	12	1.8235				
	B	12	1.8148				
	C	12	1.8137				
	D	12	1.7556	1.0825	0.0003	3	0.0001
Residuals		-	-	-	0.0003	41	0.0000073
Total		48	7.2076	1.0823	0.4822	47	-

$T = 7.2076$

$N = 48$

$T^2/N = 1.0822$

$\Sigma_x^2 = 1.5644$

PHASE II

TABLE XIV

Contact Resistance

5% v/v B-Propiolactone in Distilled Water

Replicate Specimen	Meas.	Subject k: Before Treatment ohms	%RH	Temp.°F	After Treatment ohms	%RH	Temp.°F	Change percent	Subject k': Before Treatment ohms	%RH	Temp.°F	After Treatment ohms	%RH	Temp.°F.	Change percent
1	a	0.000681	60	76	0.00088	58	75	29.4	0.00127	60	71	0.00120	57	72	-5.5
1	b	0.000700			0.00089			27.1	0.00127			0.00120			-5.5
2	a	0.000821			0.00089			8.5	0.00166			0.00161			-3.0
2	b	0.000847			0.00090			5.9	0.00166			0.00161			3.0
Cont.1	a	0.000938			0.000962			2.6	0.00100*			0.00100*			0.0
Cont.1	b	0.000938			0.000961			2.5	0.00100*			0.00100*			0.0
Cont.2	a	0.00120			0.00120*			0	0.00120			0.00120*			0.0
Cont.2	b	0.00120			0.00120*			0	0.00120			0.00120*			0.0
5% v/v B-Propiolactone in Solvent M-17															
1	a	0.000881	58	74	0.001085	58	74	23.2	0.00409	60	71	0.00151	57	72	-63.1
1	b	0.000881			0.001089			23.6	0.00409			0.00151			-63.1
2	a	0.00119			0.001100			7.6	0.00130			0.00120			-21.5
2	b	0.00119			0.001093			8.4	0.00130			0.00102			-21.5
Cont.1	a	0.000920			0.000970			5.4	0.00100			0.00100*			0.0
Cont.1	b	0.000920			0.000963			4.7	0.00100			0.00100*			0.0
Cont.2	a	0.00120			0.00120*			0.0	0.00120			0.00120*			0.0
Cont.2	b	0.00120			0.00120*			0.0	0.00120			0.00120*			0.0

PHASE II

TABLE XIV (Cont'd.)
Contact Resistance

5% v/v Ethylene Imine in Trichloroethylene

Replicate Specimen	Meas.	Subject k: Before Treatment ohms	%RH	T. °F	Subject k: After Treatment ohms	%RH	T. °F.	Change percent	Subject k': Before Treatment ohms	%RH	T. °F.	Subject k': After Treatment ohms	%RH	T. °F.	Change percent
1	a	0.000808	58	74	0.00103	57	73	27.2	0.00253	60	71	0.00149	57	72	-41.1
1	b	0.000811			0.00100			23.4	0.00253			0.00149			-41.1
2	a	0.000887			0.00121			36.1	0.00177			0.00298			68.4
2	b	0.000882			0.00121			37.4	0.00177			0.00298			68.4
Cont.1	a	0.000963			0.000970*			0.7	0.00100			0.00100*			0.0
Cont.1	b	0.000965			0.000964*			-0.1	0.00100			0.00100*			0.0
Cont.2	a	0.00121			0.00121*			0.0	0.00120			0.00120*			0.0
Cont.2	b	0.00121			0.00121*			0.0	0.00120			0.00120*			0.0
5% w/v Formaldehyde in Methanol															
1	a	0.00081	56	76	0.00138	59	75	70.3	0.00220	57	72	0.00117	57	72	-46.8
1	b	0.00078			0.00138			76.9	0.00220			0.00117			-46.8
2	a	0.00079			0.00190			139.24	0.00173			0.00119			-31.2
2	b	0.00079			0.00188			139.24	0.00173			0.00119			-31.2
Cont.1	a	0.00087			0.00093*			6.9	0.00100			0.00100*			0.0
Cont.1	b	0.00087			0.00093*			6.9	0.00100			0.00100*			0.0
Cont.2	a	0.00111			0.00118*			6.3	0.00120			0.00120*			0.0
Cont.2	b	0.00106			0.00108*			1.9	0.00120			0.00120*			0.0

* Not treated, these are repeat measurements only.

\+ a and b differ in the way in which the specimen was attached to the Kelvin bridge. The standard deviation of the Control 1 readings is 25.6×10^{-6} ohms. and of the Control 2 readings is 55.6×10^{-6} ohms. The difference in the variations of the readings is not significant.

PHASE II

TABLE XIV (Cont'd.)

Contact Resistance

5% v/v β-Propiolactone in Distilled Water

Replicate	Pin No.	Subject o							Subject o'						
		Before Treatment			After Treatment			Change	Before Treatment			After Treatment			Change
		ohms	%RH	Temp. °F	ohms	%RH	Temp. °F	Percent	ohms	%RH	Temp. °F	ohms	%RH	Temp. °F	Percent
1	2	0.00173	62	73	0.00215	59	76	24.3	0.00255	67	75	0.00255	58	74	0.00
	3	0.00177			0.00208			17.5	0.00258			0.00260			0.78
	4	0.00188			0.00248			31.9	0.00212			0.00214			0.94
	5	0.00170			0.00223			31.2	0.00229			0.00237			3.49
	6	0.00177			0.00200			11.5	0.00250			0.00248			- 0.80
2	2	0.00168			0.00179			6.5	0.00258			0.00267			3.49
	3	0.00212			0.00219			3.3	0.00239			0.00243			1.67
	4	0.00180			0.00206			14.4	0.00240			0.00242			0.83
	5	0.00158			0.00189			19.6	0.00255			0.00255			0.00
	6	0.00179			0.00206			15.1	0.00249			0.00248			- 0.40
Cont.1	2	0.00170			0.00189			11.2	0.00248			0.00250			0.80
	3	0.00170			0.00172			1.2	0.00229			0.00261			13.97
	4	0.00190			0.00196			3.2	0.00244			0.00250			4.17
	5	0.00168			0.00168			0.0	0.00248			0.00242			- 2.42
	6	0.00216			0.00194			-10.2	0.00232			0.00240			3.45
Cont.2	2	0.00165			0.00172			4.2	0.00230			0.00237			3.04
	3	0.00157			0.00157			0.0	0.00247			0.00243			- 1.62
	4	0.00193			0.00199			3.1	0.00260			0.00258			- 0.77
	5	0.00157			0.00158			0.6	0.00238			0.00247			3.78
	6	0.00197			0.00211			7.1	0.00258			0.00260			0.78

PHASE II

TABLE XIV (Cont'd)

Contact Resistance

5% v/v B-Propiolactone in Solvent M-17 *

Replicate	Pin No.	Subject o							Subject o'						
		Before Treatment			After Treatment			Change	Before Treatment			After Treatment			Change
		ohms	%RH	Temp. °F	ohms	%RH	Temp. °F	Percent	ohms	%RH	Temp. °F	ohms	%RH	Temp. °F	Percent
1	2	0.00180	62	75	0.00165	56	76	- 9.09	0.00245	56	76	0.00244	56	75	- 0.41
	3	0.00185			0.00160			-15.62	0.00260			0.00259			- 0.39
	4	0.00164			0.00163			- 0.61	0.00255			0.00257			0.78
	5	0.00167			0.00168			0.60	0.00235			0.00248			5.24
	6	0.00177			0.00183			3.28	0.00245			0.00241			- 1.66
2	2	0.00168			0.00163			- 3.07	0.00250			0.00259			1.57
	3	0.00180			0.00183			1.64	0.00248			0.00252			1.59
	4	0.00184			0.00179			- 2.79	0.00250			0.00253			1.18
	5	0.00182			0.00179			- 1.68	0.00252			0.00258			2.33
	6	0.00167			0.00163			- 2.45	0.00242			0.00245			1.22
Cont.1	2	0.00182			0.00172			- 5.81	0.00239			0.00237			- 0.84
	3	0.00162			0.00178			8.99	0.00235			0.00238			1.26
	4	0.00189			0.00196			3.57	0.00236			0.00236			0.00
	5	0.00189			0.00173			- 9.25	0.00232			0.00233			0.43
	6	0.00182			0.00180			- 1.11	0.00239			0.00238			- 0.42
Cont.2	2	0.00170			0.00170			0.00	0.00225			0.00223			- 0.90
	3	0.00158			0.00157			- 0.64	0.00253			0.00243			- 4.12
	4	0.00188			0.00189			0.53	0.00257			0.00260			1.15
	5	0.00157			0.00158			0.63	0.00242			0.00250			3.20
	6	0.00193			0.00195			1.03	0.00250			0.00258			3.10

* These connectors separated during exposure to the sterilant. The test was repeated using new connectors. The results of the repeat test follow.

PHASE II

TABLE XIV (Cont'd.)

Contact Resistance

5% v/v B-Propiolactone in Solvent M-17

Replicate	Pin No.	Subject o							Subject o'						
		Before Treatment			After Treatment			Change	Before Treatment			After Treatment			Change
		ohms	%RH	Temp.°F	ohms	%RH	Temp.°F	Percent	ohms	%RH	Temp.°F	ohms	%RH	Temp.°F	Percent
1	2	0.00181	57	72	0.00179	66	76	- 1.1	0.00255	57	72	0.00250	66	77	- 2.0
	3	0.00195			0.00171			-12.3	0.00239			0.00256			7.1
	4	0.00176			0.00180			2.3	0.00261			0.00240			- 8.0
	5	0.00202			0.00200			- 1.0	0.00269			0.00248			- 7.8
	6	0.00190			0.00190			0.0	0.00248			0.00226			- 8.9
2	2	0.00281			0.00185			-34.2	0.00241			0.00242			0.4
	3	0.00383			0.00168			-56.1	0.00281			0.00274			- 2.5
	4	0.00225			0.00193			-14.2	0.00276			0.00274			- 0.7
	5	0.00199			0.00197			- 1.0	0.00267			0.00261			- 2.2
	6	0.00188			0.00182			- 3.2	0.00251			0.00266			- 6.0
Cont.1	2	0.00193			0.00198			2.6	0.00241			0.00240			- 0.4
	3	0.00180			0.00181			0.6	0.00251			0.00251			0.0
	4	0.00191			0.00228			19.4	0.00238			0.00241			1.3
	5	0.00183			0.00181			- 1.1	0.00244			0.00246			0.8
	6	0.00191			0.00187			- 2.1	0.00235			0.00238			1.3
Cont.2	2	0.00166			0.00176			6.0	0.00245			0.00240			- 2.0
	3	0.00154			0.00158			2.6	0.00241			0.00243			0.8
	4	0.00194			0.00189			- 2.6	0.00250			0.00256			2.4
	5	0.00158			0.00165			4.4	0.00242			0.00243			0.4
	6	0.00213			0.00191			-10.3	0.00263			0.00262			- 0.4

PHASE II

TABLE XIV (Cont'd)

Contact Resistance

5% v/v Ethylene Imine in Trichloroethylene

Replicate	Pin No.	Subject o							Subject o'						
		Before Treatment			After Treatment			Change	Before Treatment			After Treatment			Change
		ohms	%RH	Temp. °F	ohms	%RH	Temp. °F	Percent	ohms	%RH	Temp. °F	ohms	%RH	Temp. °F	Percent
1	2	0.00156	60	74	0.00483	58	74	209.6	0.00229	60	75	0.00250	58	74	9.2
	3	0.00170			0.00250			47.1	0.00258			0.00260			0.77
	4	0.00160			0.00251			56.9	0.00251			0.00250			- 0.4
	5	0.00131			0.00349			166.4	0.00232			0.00252			8.6
	6	0.00174			0.00213			22.4	0.00248			0.00250			0.8
2	2	0.00195			0.00245			25.6	0.00243			0.00269			10.7
	3	0.00170			0.00191			12.4	0.00258			0.00270			4.7
	4	0.00186			0.00226			21.5	0.00248			0.00238			- 4.0
	5	0.00157			0.00180			14.6	0.00239			0.00250			4.6
	6	0.00193			0.00539			179.3	0.00253			0.00245			- 3.2
Cont. 1	2	0.00180			0.00188			4.44	0.00251			0.00250			- 0.4
	3	0.00172			0.00182			5.81	0.00239			0.00252			5.4
	4	0.00192			0.00188			- 2.08	0.00243			0.00238			- 2.1
	5	0.00169			0.00177			4.73	0.00247			0.00247			0.0
	6	0.00213			0.00182			14.62	0.00233			0.00240			3.0
Cont. 2	2	0.00171			0.00172			0.58	0.00228			0.00228			0.0
	3	0.00157			0.00157			0.00	0.00236			0.00247			4.7
	4	0.00187			0.00172			- 8.72	0.00260			0.00253			- 2.7
	5	0.00159			0.00158			- 0.62	0.00246			0.00246			0.0
	6	0.00188			0.00199			5.85	0.00259			0.00258			- 0.4

PHASE II

TABLE XIV (concl.)

Contact Resistance

5% w/v Formaldehyde in Methanol

Replicate	Pin No.	Subject o							Subject o'						
		Before Treatment			After Treatment			Change	Before Treatment			After Treatment			Change
		ohms	%RH	Temp. °F	ohms	%RH	Temp. °F	Percent	ohms	%RH	Temp. °F	ohms	%RH	Temp. °F	Percent
1	2	0.00199	58	74	0.00185	57	73	-7.04	0.00230	58	74	0.00252	61	73	9.57
	3	0.00191			0.00172			-9.95	0.00250			0.00249			- 0.40
	4	0.00190			0.00166			-12.63	0.00240			0.00249			3.75
	5	0.00158			0.00149			-5.70	0.00241			0.00230			- 4.56
	6	0.00190			0.00190			0.00	0.00241			0.00248			2.90
2	2	0.00170			0.00174			2.35	0.00254			0.00258			1.57
	3	0.00169			0.00185			9.47	0.00250			0.00249			- 0.40
	4	0.00173			0.00190			9.83	0.00241			0.00240			- 0.41
	5	0.00164			0.00190			15.85	0.00225			0.00224			- 0.44
	6	0.00172			0.00198			15.12	0.00224			0.00227			1.34
Cont.1	2	0.00199			0.00194			-2.51	0.00240			0.00240			0.00
	3	0.00184			0.00172			-6.52	0.00253			0.00255			0.79
	4	0.00173			0.00182			5.20	0.00240			0.00239			- 0.42
	5	0.00178			0.00181			1.69	0.00230			0.00250			8.70
	6	0.00199			0.00191			-4.02	0.00235			0.00232			- 1.28
Cont.2	2	0.00168			0.00169			0.60	0.00226			0.00228			0.88
	3	0.00156			0.00156			0.00	0.00247			0.00247			0.00
	4	0.00180			0.00192			6.67	0.00253			0.00252			- 0.40
	5	0.00158			0.00158			0.00	0.00248			0.00243			- 2.02
	6	0.00213			0.00201			-7.80	0.00259			0.00259			0.00

PHASE II

TABLE XV

Summary of Table XIV

Relative Change in Contact Resistance, percent

Subject	Replicate	Sterilant			
		A	B	C	D
k	1a	29.4	23.2	27.2	58.0
	1b	27.1	23.6	23.4	64.1
	2a	8.5	-7.6	36.1	139.2
	2b	5.9	-8.4	37.4	139.2
k'	1a	-5.5	-63.1	-41.1	-46.8
	1b	-5.5	-63.1	-41.1	-46.8
	2a	-3.0	-21.5	68.4	-31.2
	2b	-3.0	-21.5	68.4	-31.2

PHASE II

TABLE XVI

Analysis of Variance in Relative Change in Contact Resistance of Subjects k and k'

Factor	Level	n_*	T_*	$\Sigma T_*^2/n_*$	S_*	df	MS
Subject	k k'	16 16	6263 -2876	2,968,534	2,610,042	2	1,305,021
Measurement	a b	16 16	1702 1685	358,501	9	2	4.5
Replicate	1 2	16 16	- 370 3757	890,746	532,254	2	266,127
Sterilant	A B C D	8 8 8 8	539 -1384 1787 2445	1,422,171	1,063,679	3	354,560
Residuals		-	-	-	3,552,917	22	161,596
Total		4	3387	358,492	7,758,901	31	-

T = 3387

N = 32

T^2/N = 358,492

Σx^2 = 8,117,393

PHASE II

TABLE XVII

Summary of Table XIV

CONTACT RESISTANCE, ohms

Subject	Replicate	Pin No.	Before Treatment					After Treatment				
			A	B*	B	C	D	A	B*	B	C	D
O	1	2	0.00173	0.00180	1.81	0.00156	0.00199	0.00215	0.00165	1.79	0.00483	0.00185
		3	0.00177	0.00185	1.95	0.00170	0.00191	0.00208	0.00160	1.71	0.00250	0.00172
		4	0.00188	0.00164	1.76	0.00160	0.00190	0.00248	0.00163	1.80	0.00251	0.00166
		5	0.00170	0.00167	2.02	0.00131	0.00158	0.00223	0.00168	2.00	0.00349	0.00149
		6	0.00177	0.00177	1.90	0.00174	0.00190	0.00200	0.00183	1.90	0.00213	0.00190
	2	2	0.00168	0.00168	2.81	0.00195	0.00170	0.00179	0.00163	1.85	0.00245	0.00174
		3	0.00212	0.00180	3.83	0.00170	0.00169	0.00219	0.00183	1.68	0.00191	0.00185
		4	0.00180	0.00184	2.25	0.00186	0.00173	0.00206	0.00179	1.95	0.00226	0.00190
		5	0.00158	0.00182	1.99	0.00157	0.00164	0.00189	0.00179	1.97	0.00180	0.00190
		6	0.00179	0.00167	1.88	0.00193	0.00172	0.00206	0.00163	1.82	0.00539	0.00198
O'	1	2	0.00255	0.00245	2.55	0.00229	0.00230	0.00255	0.00244	2.50	0.00250	0.00252
		3	0.00258	0.00260	2.39	0.00258	0.00250	0.00260	0.00259	2.56	0.00260	0.00249
		4	0.00212	0.00255	2.61	0.00251	0.00240	0.00214	0.00257	2.40	0.00250	0.00249
		5	0.00229	0.00235	2.69	0.00232	0.00241	0.00237	0.00248	2.48	0.00252	0.00230
		6	0.00250	0.00245	2.48	0.00248	0.00241	0.00248	0.00241	2.26	0.00250	0.00248
	2	2	0.00258	0.00250	2.41	0.00243	0.00254	0.00267	0.00259	2.42	0.00269	0.00258
		3	0.00239	0.00248	2.81	0.00258	0.00250	0.00243	0.00252	2.74	0.00270	0.00249
		4	0.00240	0.00250	2.76	0.00248	0.00241	0.00242	0.00253	2.74	0.00238	0.00240
		5	0.00255	0.00252	2.67	0.00239	0.00225	0.00255	0.00258	2.61	0.00250	0.00224
		6	0.00249	0.00242	2.57	0.00253	0.00224	0.00248	0.00245	2.66	0.00245	0.00227

* The connectors separated during storage with the sterilant. The test was repeated using new connectors. The results of the second test are recorded in the columns under B.

PHASE II

TABLE XVIII

Analysis of Variance in Contact Resistance

Factor	Level	n_*	T_*	$\Sigma T_*^2/n_*$	S_*	df	MS	MS+
Subject	o	80	161.36					
	o'	80	198.60	818.48	8.66	1	8.66	11.01
Replicate	1	80	177.90					
	2	80	182.06	809.92	0.10	1	0.10	0.00
Pin	2	32	73.76					
	3	32	73.25					
	4	32	70.56					
	5	32	69.30					
	6	32	73.09	810.29	0.47	4	0.12	0.12
Treatment	Before	80	173.64					
	After	80	186.32	810.82	1.00	1	1.00	1.77
Sterilant	A	40	87.89					
	B	40	91.98					
	C	40	96.12					
	D	40	83.97	811.87	2.05	3	0.68	0.78
Residuals		-	-		28.59	149	0.19	0.162
Total		160	359.96	809.82	40.87	159	-	-

T = 359.96
N = 160
T^2/n = 809.82
Σx^2 = 850.6900

\+ Calculated using the values listed under B* in Table XVII whereas the remainder of this table is based on the values listed under B in Table

PHASE II

TABLE XIX

Contact Resistance, ohms x 10^5

Subject	Pin No.	Number of Times Contact Closed					
		1	2	3	4	5	Sum
k		86.	96	107	112	110	-
O	2	179	196	172	189	187	923
	3	182	178	184	187	179	910
	4	189	201	182	194	180	946
	5	157	159	179	183	163	841
	6	191	171	217	180	214	973
	-	898	905	934	933	923	Sum
O'	2	240	245	242	257	-	984
	3	240	232	229	262	-	963
	4	240	248	252	254	-	994
	5	245	255	241	243	-	984
	6	245	237	242	248	-	972
	-	1210	1217	1206	1264	-	Sum

PHASE II

TABLE XX

Electrical Resistance of Insulators
5% v/v B-Propiolactone in Distilled Water

Replicate	Pin No.	Voltage Sign	Subject o								Subject o'							
			Before Treatment				After Treatment				Before Treatment				After Treatment			
			Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH
Short		+	42	0.00001	76	62	43**	0.00001	74	58								
		-	42	0.00001	75	59	43**	0.00001	74	58								
		+	43	0.00001	75	62	43**	0.00001	76	59								
1	2	+	2.0	1	76	62	3.0	1	74	58	6.0	1	75	62	39.0	0.00001	76	59
	2	-	1.5				2.5				6.0				40.0	0.00001		
	3	+	2.0				1.0				10.0				36.0	0.00001		
	3	-	1.0				1.0				8.5				38.0	0.00001		
	4	+	1.0				1.0				8.0				38.5	0.00001		
	4	-	1.0				1.0				7.0				40.0	0.00001		
	5	+	1.0				1.0				6.0				21.5+	0.0001		
	5	-	1.0				1.0				5.0				24.5	0.0001		
	6	+	1.0				1.5				7.5				42+2±	0.0001		
	6	-	1.0				1.0				7.0				25-35#	0.0001		
2	2	+	1.5	1	76	62	1.0	1	74	58	3.0	1	75	62	15±	0.0001	76	59
	2	-	1.0				1.0				2.0				15	0.001		
	3	+	2.0				1.5				10.5				103	0.001		
	3	-	2.0				1.5				9.0				62	0.001		
	4	+	1.5				1.5				3.5				75+	0.001		
	4	-	1.0				1.0				3.0				80	0.001		
	5	+	1.5				2.0				3.0				74	0.001		
	5	-	1.5				2.0				2.5				62	0.001		
	6	+	1.0				2.5				3.0				64	0.001		
	6	-	1.0				2.0				3.0				55	0.001		

PHASE II

TABLE XX (Cont'd)

Electrical Resistance of Insulators
5% v/v B-Propiolactone in Distilled Water

Replicent	Pin No.	Voltage Sign	Subject c								Subject o'							
			Before Treatment				After Treatment				Before Treatment				After Treatment			
			Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH
Cont. 1	2	+	2.0	1	76	62	2.5	1	74	58	3.5	1	75	62	6.5	1	75	58
	2	-	2.0				2.0				3.0				4.5			
	3	+	1.0				1.0				6.5				5.5			
	3	-	1.0				0.5				5.5				4.5			
	4	+	1.5				2.0				6.0				5.0			
	4	-	1.0				2.0				5.0				4.5			
	5	+	1.0				3.0				4.5				7.0			
	5	-	1.0				2.0				3.0				5.0			
	6	+	1.0				1.0				5.0				4.0			
	6	-	1.0				0.5				4.0				3.0			
Cont. 2	2	+	1.5	1	75	59	2.0	1	74	58	3.0	1	75	62	5.0	1	75	58
	2	-	1.0				1.5				2.5				3.0			
	3	+	1.0				2.5				5.0				5.0			
	3	-	1.0				2.0				4.0				3.0			
	4	+	1.0				3.0				3.0				4.0			
	4	-	1.0				2.0				2.5				2.0			
	5	+	1.0				1.0				3.0				3.5			
	5	-	1.0				0.5				2.5				1.5			
	6	+	1.5				2.0				5.0				3.0			
	6	-	1.0				1.0				4.0				3.0			

PHASE II

TABLE XX (Cont'd.)

Electrical Resistance of Insulators

5% v/v B-Propiolactone in Solvent M-17*

Replicate	Pin No.	Voltage Sign	Subject o								Subject o'							
			Before Treatment				After Treatment				Before Treatment				After Treatment			
			Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH
Short		+	42	0.00001	76	62												
		-	42	0.00001	76	62												
		+	43	0.0001	76	56												
1	2	+	3.0	1	76	59	1.0	1	74	59	5.5	1	78	57	127.0	1	75	62
	2	-	3.0				0.5				4.0				126.0			
	3	+	7.0				2.0				4.0				143.5			
	3	-	6.0				1.5				3.5				142.0			
	4	+	1.5				2.5				3.0				132.0			
	4	-	1.0				2.0				3.5				129.0			
	5	+	1.0				1.5				3.5				157.0			
	5	-	1.0				1.0				3.5				157.0			
	6	+	1.0				4.0				3.5				90.0			
	6	-	1.0				5.5				4.0				85.0			
2	2	+	6.0	1	76	59	2.0	1	74	59	3.0	1	78	57	25.5	1	75	59
	2	-	8.5				1.0				3.0				23.0			
	3	+	1.0				2.0				3.0				35.0			
	3	-	1.0				2.0				3.0				30.0			
	4	+	1.0				1.0				4.0				22.5			
	4	-	0.5				1.0				2.0				20.0			
	5	+	3.0				1.0				3.0				22.0			
	5	-	2.0				0.5				3.0				20.5			
	6	+	1.0				1.5				3.0				22.5			
	6	-	1.0				1.0				3.0				20.5			

PHASE II

Electrical Resistance of Insulators

5% v/v B-Propiolactone ir Solvent M-17*

Replicate	Pin No.	Voltage Sign	Subject o								Subject o							
			Before Treatment				After Treatment				Before Treatment				After Treatment			
			Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH
Cont. 1	2	+	2.0	1	74	62	1.5	1	75	59	3.5	1	78	57	3.5	1	75	62
	2	-	2.0				0.5				3.5				2.5			
	3	+	2.5				1.0				4.5				3.5			
	3	-	2.5				1.0				4.0				3.0			
	4	+	1.0				1.0				4.0				4.0			
	4	-	1.0				0.5				7.5				3.5			
	5	+	2.0				2.0				11.5				3.5			
	5	-	2.0				1.0				8.0				3.0			
	6	+	1.0				1.0				5.0				3.5			
	6	-	1.0				1.0				4.5				3.0			
Cont. 2	2	+	3.0	1	74	62	1.0	1	75	59	3.0	1	78	57	3.5	1	75	62
	2	-	2.0				0.5				3.5				4.0			
	3	+	4.0				2.0				2.5				4.0			
	3	-	3.0				0.5				3.0				3.0			
	4	+	1.5				2.0				3.0				3.5			
	4	-	1.0				0.5				3.0				2.5			
	5	+	1.5				1.5				3.0				3.0			
	5	-	1.0				1.0				2.5				2.5			
	6	+	1.0				1.0				3.0				3.5			
	6	-	1.0				0.5				3.0				2.0			

PHASE II

TABLE XX (Cont'd.)

Electrical Resistance of Insulators

5% B-Propiolactone in Solvent M-17

Replicate	Pin No.	Voltage Sign	Subject o								Subject o'							
			Before Treatment				After Treatment				Before Treatment				After Treatment			
			Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. Defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH
Short		+	42	0.00001	76	62												
		-	42	0.00001	76	62												
		+	43	0.00001	76	56												
1	2	+	1.0	1	72	57	2.0	1	76	66	3.0	1	72	57	12.0	1	77	66
	2	-	0.5				1.0				2.5				12.0			
	3	+	1.0				2.0				3.0				16.5			
	3	-	0.5				0.5				3.0				17.0			
	4	+	0.5				1.0				3.5				25.0			
	4	-	0.5				0.5				3.0				26.5			
	5	+	0.5				1.0				2.0				25.0			
	5	-	0.5				0.5				1.5				16.0			
	6	+	0.5				2.0				2.0				17.5			
	6	-	0.5				1.5				2.0				18.5			
2	2	+	0.5				2.0				2.0	1	72	57	26.0	1	77	66
	2	-	0.5				2.0				2.0				27.0			
	3	+	0.5				2.0				2.5				26.0			
	3	-	0.5				2.0				2.5				26.5			
	4	+	0.5				0.5				2.0				30.5			
	4	-	0.5				1.0				2.0				30.5			
	5	+	0.5				1.0				2.0				25.0			
	5	-	0.5				1.0				1.5				24.5			
	6	+	0.5				1.0				3.0				25.5			
	6	-	0.5				1.5				3.0				25.5			

PHASE II

TABLE XX (Cont'd.)

Electrical Resistance of Insulators

5% B-Propiolactone in Solvent M-17

Replicate	Pin No.	Voltage Sign	Subject o								Subject o'							
			Before Treatment				After Treatment				Before Treatment				After Treatment			
			Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. Defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH
Cont. 1	2	+	1.0	1	72	57	1.0	1	76	66	2.5	1	73	61	6.0	1	77	66
	2	-	1.0				1.0				2.5				6.5			
	3	+	2.0				2.0				2.0				4.5			
	3	-	2.0				2.0				2.0				5.0			
	4	+	2.0				2.5				3.5				5.5			
	4	-	2.0				3.0				3.0				6.0			
	5	+	1.5				3.0				3.0				3.0			
	5	-	1.5				4.0				3.0				4.0			
	6	+	0.5				0.5				3.0				3.5			
	6	-	0.5				1.5				3.5				4.5			
Cont. 2	2	+	2.0	1	72	62	3.5	1	77	66	1.5	1	73	61	2.5	1	77	66
	2	-	1.5				3.0				1.5				3.0			
	3	+	1.0				5.5				2.0				4.5			
	3	-	1.0				4.5				2.0				5.0			
	4	+	2.0				4.0				2.0				3.0			
	4	-	2.0				4.0				2.0				5.0			
	5	+	4.0				5.0				2.0				2.0			
	5	-	3.0				4.0				2.0				3.0			
	6	+	1.0				1.5				2.0				2.0			
	6	-	0.5				1.5				2.0				3.5			

PHASE II

TABLE XX (Cont.)

Electrical Resistance of Insulators

5% v/v Ethylene Imine in Trichloroethylene

Replicate	Pin No.	Voltage Sign	Subject o								Subject o'							
			Before Treatment				After Treatment				Before Treatment				After Treatment			
			Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH
Short		+	43	0.00001	76	59	44**	0.00001	74	58								
		-	43	0.00001	76	59	43**	0.00001	72	57								
		+	43	0.00001	75	62	43**	0.00001	74	58								
1	2	+	4.0	1	76	59	2.0	1	74	58	3.0	1	76	59	27	1	72	57
	2	-	3.0				1.5				3.0				27			
	3	+	3.5				2.0				5.0				30			
	3	-	3.0				0.5				4.0				29			
	4	+	3.5				1.0				4.0				50			
	4	-	3.0				2.0				4.0				47			
	5	+	3.0				0.5				3.0				37			
	5	-	3.0				1.0				3.0				35			
	6	+	3.0				1.5				4.0				47			
	6	-	3.0				1.5				3.5				45			
2	2	+	4.0	1	76	59	1.5	1	74	58	4.0	1	76	59	20	1	72	57
	2	-	3.5				2.0				4.0				20			
	3	+	4.0				1.0				4.0				27			
	3	-	4.0				1.0				4.0				25			
	4	+	1.5				1.0				4.0				26			
	4	-	1.5				0.5				5.0				24			
	5	+	1.5				1.5				3.0				14			
	5	-	2.0				1.0				3.0				12			
	6	+	1.0				1.0				3.0				15			
	6	-	2.0				0.5				4.0				13			

PHASE II

TABLE XX (Cont.)

Electrical Resistance of Insulators
5% v/v Ethylene Imine in Trichloroethylene

Replicate	Pin No.	Voltage Sign	Subject o Before Treatment Galv. defl. mm.	Shunt Setting	°T,F	%RH	Subject o After Treatment Galv. defl. mm.	Shunt Setting	°T,F	%RH	Subject o' Before Treatment Galv. Defl. mm.	Shunt Setting	°T,F	%RH	Subject o' After Treatment Galv. defl. mm.	Shunt Setting	°T,F	%RH
Cont. 1	2	+	2.0	1	76	59	3.0	1	74	58	3.5	1	75	62	3.0	1	74	58
	2	-	2.5				1.5				3.0				2.0			
	3	+	1.0				1.5				6.5				4.5			
	3	-	2.0				0.5				5.5				3.5			
	4	+	1.0				1.5				6.0				4.0			
	4	-	1.0				0.5				5.0				3.5			
	5	+	1.0				3.0				4.5				3.0			
	5	-	1.5				2.0				3.0				2.0			
	6	+	1.0				1.5				5.0				4.0			
	6	-	1.0				0.5				4.0				3.0			
Cont. 2	2	+	1.0	1	76	59	3.0	1	72	57	3.0	1	75	62	3.0	1	74	58
	2	-	1.0				2.0				2.5				2.0			
	3	+	1.0				3.5				5.0				3.0			
	3	-	1.5				2.0				4.0				2.0			
	4	+	1.0				2.0				3.0				2.0			
	4	-	0.5				2.0				2.5				2.0			
	5	+	2.0				0.5				3.0				5.0			
	5	-	1.0				1.0				2.5				3.5			
	6	+	2.0				1.0				5.0				3.0			
	6	-	1.0				1.0				4.0				2.0			

PHASE II

TABLE XX (Cont.)

Electrical Resistance of Insulators

5% w/v Formaldehyde in Methanol

Replicate	Pin No.	Voltage Sign	Subject o								Subject o'							
			Before Treatment				After Treatment				Before Treatment				After Treatment			
			Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH	Galv. Defl. mm.	Shunt Setting	T°F	%RH	Galv. defl. mm.	Shunt Setting	T°F	%RH
Short		+	43	0.00001	74	58	44**	0.00001	73	61								
		-	44	0.00001	74	58	44**	0.00001	74	61								
		+	43	0.00001	74	58	43**	0.00001	74	61								
1	2	+	1.0	1	74	58	1.0	1	73	61	4.0	1	74	58	57	0.001	74	61
	2	-	0.5				1.0				4.0				60			
	3	+	1.0				9.0				4.5				50			
	3	-	0.5				6.5				4.0				33			
	4	+	0.5				4.0				5.0				47			
	4	-	0.5				3.0				4.0				53			
	5	+	1.0				1.5				3.0				41			
	5	-	1.0				1.0				2.0				46			
	6	+	1.0				1.0				4.5				30			
	6	-	0.5				0.5				3.5				31			
2	2	+	1.0	1	74	58	1.0	1	73	61	3.0	1	74	58	29	0.001	74	61
	2	-	0.5				0.5				2.0				37			
	3	+	1.0				2.0				3.0				25			
	3	-	0.5				1.0				2.5				30			
	4	+	0.5				1.0				3.5				28			
	4	-	0.5				0.5				2.5				45			
	5	+	1.0				3.0				2.5				19			
	5	-	1.0				2.0				2.0				22			
	6	+	1.0				1.0				3.0				31			
	6	-	1.0				0.5				2.5				26			

PHASE II

TABLE XX (Cont.)

Electrical Resistance of Insulators

5% w/v Formaldehyde in Methanol

Replicate	Pin No.	Voltage Sign	Subject o Before Treatment: Galv. defl. mm.	Shunt Setting	T°F	%RH	Subject o After Treatment: Galv. defl. mm.	Shunt Setting	T°F	%RH	Subject o' Before Treatment: Galv. defl. mm.	Shunt Setting	T°F	%RH	Subject o' After Treatment: Galv. defl. mm.	Shunt Setting	T°F	%RH
Cont. 1	2	+	3.0	1	74	58	2.5	1	73	61	2.5	1	74	58	2.0	1	74	61
	2	-	2.5				2.0				2.0				2.0			
	3	+	1.0				2.5				7.0				6.0			
	3	-	1.0				1.0				5.0				5.5			
	4	+	3.5				4.0				3.0				5.0			
	4	-	2.0				3.0				3.0				5.0			
	5	+	3.0				1.5				2.5				4.0			
	5	-	2.5				0.5				2.0				4.0			
	6	+	0.5				1.0				3.0				3.5			
	6	-	0.5				0.5				3.0				3.0			
Cont. 2	2	+	3.0	1	74	58	3.0	1	74	61	2.0	1	74	58	3.0	1	74	61
	2	-	2.0				1.5				2.0				3.0			
	3	+	3.0				2.0				3.0				5.5			
	3	-	3.0				1.0				3.5				5.0			
	4	+	3.0				1.0				2.0				5.0			
	4	-	2.0				0.5				3.0				4.5			
	5	+	3.5				0.5				2.0				2.5			
	5	-	2.0				1.0				2.0				2.5			
	6	+	1.5				0.5				2.0				3.0			
	6	-	0.5				1.0				3.0				3.0			

** These are replicate measurements. The after treatment heading does not apply.

* Connectors came apart during exposure to the sterila t. The measurements were repeated and the results reported in the section following.

+ As the galvanometer approached rest point it often jumped suddenly. The rest point often changed after these spurts

* Same phenomenon as that described in preceeding footnote. The galvanometer never did come to rest.

Same as preceeding footnote.

≠ After the galvanometer stopped oscillating the deflection dropped slowly from 30 to 15 where it rested.

≠ The reading is the initial rest point before the downward drift in the deflection.

PHASE II

TABLE XXI

Logarithm of Electrical Resistance of Insulators, $\log_{10}$ ohms

SUBJECT O

Replicate	Pin No.	Voltage	Sterilant									
			Before Treatment					After Treatment				
			A	B*	B	C	D	A	B*	B	C	D
1	2	+	12.34	12.15	12.63	12.04	12.63	12.15	12.63	12.34	12.34	12.63
		−	12.46	12.15	12.93	12.14	12.93	12.23	12.93	12.63	12.46	12.63
	3	+	12.34	11.79	12.63	12.08	12.63	12.63	12.34	12.34	12.34	11.68
		−	12.63	11.86	12.93	12.15	12.93	12.63	12.46	12.93	12.93	11.82
	4	+	12.63	12.46	12.93	12.08	12.93	12.63	12.23	12.63	12.63	12.04
		−	12.63	12.63	12.93	12.15	12.93	12.63	12.34	12.93	12.34	12.15
	5	+	12.63	12.63	12.93	12.15	12.63	12.63	12.46	12.63	12.93	12.46
		−	12.63	12.63	12.93	12.15	12.63	12.63	12.63	12.93	12.63	12.63
	6	+	12.63	12.63	12.93	12.15	12.63	12.46	12.04	12.34	12.46	12.63
		−	12.63	12.63	12.93	12.15	12.93	12.63	11.89	12.46	12.46	12.93
2	2	+	12.46	11.86	12.93	12.04	12.63	12.63	12.34	12.34	12.46	12.63
		−	12.63	11.71	12.93	12.08	12.93	12.63	12.63	12.34	12.34	12.93
	3	+	12.34	12.63	12.93	12.04	12.63	12.46	12.34	12.34	12.63	12.34
		−	12.34	12.63	12.93	12.04	12.93	12.46	12.34	12.34	12.63	12.63
	4	+	12.46	12.63	12.93	12.46	12.93	12.46	12.63	12.93	12.63	12.63
		−	12.63	12.93	12.93	12.46	12.93	12.63	12.63	12.63	12.93	12.93
	5	+	12.46	12.15	12.93	12.46	12.63	12.34	12.63	12.63	12.46	12.14
		−	12.46	12.34	12.93	12.34	12.63	12.34	12.93	12.63	12.63	12.34
	6	+	12.63	12.63	12.93	12.63	12.63	12.23	12.46	12.63	12.63	12.63
		−	12.63	12.63	12.93	12.34	12.63	12.34	12.63	12.46	12.93	12.93

SUBJECT O'

1	2	+	11.86	11.89	12.15	12.15	12.04	5.00	10.53	11.56	11.20	7.87
		−	11.86	12.04	12.23	12.15	12.04	4.88	10.53	11.56	11.20	7.85
	3	+	11.63	12.04	12.15	11.93	11.98	5.28	10.48	11.42	11.15	7.92
		−	11.70	12.08	12.15	12.04	12.04	5.11	10.48	11.40	11.18	7.91
	4	+	11.73	12.15	12.08	12.04	11.93	5.08	10.52	11.23	10.93	7.95
		−	11.79	12.08	12.15	12.04	12.04	4.88	10.52	11.20	10.96	7.90
	5	+	11.86	12.08	12.34	12.15	12.15	7.28	10.43	11.23	11.08	8.00
		−	11.93	12.08	12.46	12.15	12.34	7.23	10.43	11.48	11.08	7.96
	6	+	11.76	12.08	12.34	12.04	11.98	6.96	10.68	11.40	10.96	8.15
			11.78	12.04	12.34	12.08	12.08	7.21	10.71	11.36	10.98	8.15
2	2	+	12.15	12.15	12.34	12.04	12.15	7.45	11.23	11.23	11.34	8.18
		−	12.34	12.15	12.34	12.04	12.34	8.46	11.28	11.20	11.34	8.07
	3	+	11.61	12.15	12.23	12.04	12.15	7.61	11.08	11.23	11.20	8.23
		−	11.68	12.15	12.23	12.04	12.23	7.83	11.15	11.20	11.28	8.15
	4	+	12.08	12.04	12.34	12.04	12.08	7.75	11.28	11.15	11.20	8.18
		−	12.15	12.34	12.34	11.93	12.23	7.72	11.34	11.15	11.26	7.98
	5	+	12.15	12.14	12.34	12.15	12.23	7.76	11.30	11.23	11.49	8.36
		−	12.23	12.15	12.46	12.15	12.34	7.83	11.32	11.26	11.56	8.28
	6	+	12.15	12.15	12.15	12.15	12.15	7.82	11.30	11.23	11.46	8.15
		−	12.15	12.15	12.15	12.04	12.23	7.89	11.32	11.23	11.52	8.20

* The connectors separated during storage with the sterilant. The test was repeated using new connectors. The results of the second test are recorded in the columns under B.

PHASE II

TABLE XXII

Analysis of Variance in Electrical Resistance of Insulators

Factor	Level	n_*	T_*	$\sum T_*^2/n_*$	S_*	df	MS	MS+
Subject	0	160	2010.74					
	0'	160	1716.78	43,690.05	270.04	1	270.04	268.21
Replicate	1	160	1848.63					
	2	160	1878.89	43,422.87	2.86	1	2.86	5.58
Voltage	+	160	1858.65					
	-	160	1868.87	43,420.34	0.33	1	0.33	0.35
Pin No.	2	64	743.05					
	3	64	739.51					
	4	64	744.20					
	5	64	751.04					
	6	64	749.72	43,421.45	1.44	4	0.36	0.42
Treatment	Before	160	1976.06					
	After	160	1751.46	43,577.65	157.64	1	157.64	152.74
Sterilant	A	80	875.98					
	B	80	980.69					
	C	80	961.63					
	D	80	909.22	43,506.33	86.32	3	28.77	20.96
Residuals		-	-	-	401.49	308	1.30	1.34
Total		320	3727.52	43,420.01	920.12	319	-	-

T = 3727.52
N = 320
T^2/n = 43,420.01
Σx^2 = 44,340.1320

\+ Calculated using the values listed under B* in Table XXI whereas the remainder of this table is based on the values listed in B in Table .

PHASE II

TABLE XXIII

Solubility of Silicone Grease

5% v/v B-Propiolactone in Distilled Water

Replicate	Initial Silicone Mg.	Tare Mg.	Total Mg.	Residue Mg.
1	1000 ±10	42,420.3	42,986.0	475.2
2	1000 ±10	42,421.5	42,791.4	369.9

5% v/v B-Propiolactone in Solvent M-17

1	1000±10	44,427.9	44,619.4	191.5
2	1000±10	44,427.6	44,703.5	275.9

5% v/v Ethylene Imine in Trichloroethylene

1	1000±10	43,462.7	43,695.8	233.1
2	1000±10	43,462.5	43,533.2	70.7

5% w/v Formaldehyde in Methanol

1	1000±10	44,242.8	44,292.2	42.8
2	1000±10	44,243.2	44,275.5	32.3

PHASE II

TABLE XXIV

Analysis of Variance in Weight of Residue Extracted from Silicone Grease

Factor	Level	n_*	T_*	$\sum T^2/n_*$	S_*	df	MS
Replicate	1	4	942.6				
	2	4	748.8	362,299	4,695	3	1,565
Sterilant	A	2	845.1				
	B	2	467.4				
	C	2	303.8				
	D	2	75.1	515,296	157,692	1	157,692
Residuals		-	-	-	17,652	3	5,884
Totals		8	1691.4	357,604	180,039	7	-

$N = 8$

$T_2 = 1691.4$

$T^2/N = 357,604$

$\Sigma x^2 = 537,643$

PHASE II

TABLE XXV

Ability of Sterilant to Wet Subject (an x indicate that sterilant wet subject)

Subject \ Sterilant	A*	B	C	D
a		x	x	x
b		x	x	
c		x	x	x
d		x	x	x
e				
f				
g				x
h	x	x	x	x
i	x	x	x	x
j	x	x	x	x
k		x	x	x
l	x	x	x	x
m	x	x	x	x
n (metal)	x	x	x	x
n (rubber)		x	x	x
o	x	x	x	x
o	x	x	x	x
p	x	x	x	x
q	x	x	x	x

* A. 5% v/v B-Propiolactone/Eastman in Distilled Water
B. 5% v/v B-Propiolactone/Eastman in Solvent M-17
C. 5% v/v Ethylene Imine in Trichloroethylene
D. 5% w/v Formaldehyde in Methanol

PHASE II

TABLE XXVI

Effect of Vehicle on Sterilizing Effectiveness of Formaldehyde during a 90 minute contact in a Petri dish

1.85% w/v Formaldehyde in vehicle

Colonies of B. subtilis var. niger**

Vehicle	Replicate		Inoculum on Magnesium alloy strip		
	Dish	Plate	10^6 (10^4)*	10^7 (10^5)*	10^8 (10^6)*
Methanol	1	a	768	TNC	TNC
	1	b	576	TNC	TNC
	2	a	106	478	TNC
	2	b	186	628	TNC
Isopropanol	1	a	512	TNC	TNC
	1	b	912	TNC	TNC
	2	a	780	TNC	TNC
	2	b	868	TNC	TNC

5% w/v Formaldehyde in Vehicle

Methanol	1	a	0	0	TNC
	1	b	0	0	TNC
	2	a	TNC	TNC	TNC
	2	b	TNC	TNC	TNC
Isopropanol	1	a	182	9	291
	1	b	163	8	209
	2	a	0	0	0
-----	2	b	0	0	0

* The maximum number of colonies expected, if all spores in the inoculum developed colonies when placed on Trypticase soy agar.

** In one day on Trypticase soy agar/Hyland at 37°C.

PHASE II

TABLE XXVI (Cont.)

5% w/v Formaldehyde in Vehicle

Colonies of B. subtilis, var. niger**

Vehicle	Replicate		Inoculum on Magnesium alloy strip		
	Dish	Plate	10^6 (10^4)*	10^7 (10^5)*	10^8 (10^6)*
Methanol	1	a	0	0	TNC
	1	b	0	0	TNC
	2	a	TNC	TNC	TNC
	2	b	TNC	TNC	TNC
Isopropanol	1	a	304	35	334
	1	b	294	36	373
	2	a	2	34	35
	2	b	2	23	32

* The maximum number of colonies expected, if all spores in the inoculum developed colonies when placed on Trypticase soy agar.

** In three days on Trypticase soy agar/Hyland at 37°C, same plates as were reported in immediately preceding section of this table.

PHASE II

TABLE XXVII

Effect of Vehicle on Sterilizing Effectiveness of 5% Ethylene Imine during a 90 minute contact in a Petri dish

Colonies of B. subtilis, var. niger**

Vehicle	Replicate		Inoculum on Magnesium alloy strip		
	Dish	Plate	10^6 (10^4)*	10^7 (10^5)*	10^8 (10^6)*
Methanol	1	a	1	0	2
	1	b	3	0	7
	2	a	1	0	1
	2	b	0	1	0
	3	a	0	5	22
	3	b	0	2	30(1)
Isopropanol	1	a	TNC	628	TNC
	1	b	TNC	576	TNC
	2	a	43	448(1)	TNC
	2	b	39	346	TNC
	3	a	746	TNC	TNC
	3	b	756	TNC	TNC
	4	a	654	TNC	TNC
	4	b	732	TNC	TNC
Distilled Water	1	a	2	1	456
	1	b	0	3	564
	2	a	1	8	245
	2	b	1	10	252
5 minute exposure in Petri dish					
Methanol	1	a	285	TNC	TNC
	1	b	296	TNC	TNC
	2	a	780	TNC	TNC
	2	b	984	TNC	TNC

* The maximum number of colonies expected, if all spores in the inoculum developed colonies when placed on Trypticase soy agar.

** In one day on Trypticase soy agar/Hyland at 37°C.

PHASE II

TABLE XXVIII

Liquid Culture Sterility Test

Subject	Sterilant	Growth of *B. subtilis var. niger* population
a	A	Yes
	B	No
	C	Yes
	D	No
	D	No*
e	A	No
	B	Yes
	C	Yes
	D	No
i	B	Yes
j	B	No

* A Gram positive coccus population grew in the broth.

PHASE II

TABLE XXIX

Effect of Ratio of Sterilant to Inoculum on Sterilizing Effectiveness During a 90-Minute Contact in a Petri Dish

Colonies of B. subtilis var. niger*

Sterilant	Volume of Sterilant	Replicate		Specimen Inoculum			Bacteriostasis Inoculum		
		Strip	Plate	$1:10^6$	$1:10^7$	$1:10^8$	$1:10^4$	$1:10^5$	$1:10^6$
5% v/v B-Propiolactone in Distilled Water	1	1	a	0	0	0	102	554	TNC
			b	0	0	0			
	0.1	1	a	0	0	0	85	772	TNC
			b	0	0(2)	0			
	0.01	1	a	0	0	0	63	622	TNC
			b	0	0	0			
5% v/v Betaprone+ in Distilled Water	1	1	a	0	0	0	45(1)	504	861
			b	0	0	0			
	0.1	1	a	0	0	0	47	532	TNC
			b	0	0	0			
	0.01	1	a	0	0	0	44	496	TNC
			b	0	0	0			
5% v/v B-Propiolactone in 2% w/v Tide Water	1	1	a	0	0	0	41(1)	572	TNC
			b	0	0	0			
	0.1	1	a	0	0	0	63	388	TNC
			b	0	0	0			
	0.01	1	a	–	0	0	81(18)	376	TNC
			b	–	0	0			
5% v/v B-Propiolactone in Solvent M-17	1	1	a	0	0	0	82(3)	378	TNC
			b	0(1)	0	0			
	0.1	1	a	0	0(1)	0	85	916	TNC
			b	0	0	0			
	0.01	1	a	–	0	0	171	880	TNC
			b	–	0	0			

TABLE XXIX
(Cont.)

Effect of Ratio of Sterilant to Inoculum on Sterilizing Effectiveness During a 90-Minute Contact in a Petri Dish

Colonies of B. subtilis var. niger*

Sterilant	Volume of Sterilant	Replicate		Specimen Inoculum			Bacteriostasis Inoculum		
		Strip	Plate	$1:10^6$	$1:10^7$	$1:10^8$	$1:10^4$	$1:10^5$	$1:10^6$
• 5% v/v Ethylene Imine in Trichloroethylene	1	1	a	0	0	0	49	568	TNC
			b	0	0	0			
	0.1	1	a	0	0	610	96	TNC	TNC
			b	0	0	452			
	0.01	1	a	578(2)	213(1)	TNC	88	742	TNC
			b	242(1)	148	TNC			
5% w/v Formaldehyde In Methanol	1	1	a	21	TNC	TNC	77	786	TNC
			b	19	TNC	TNC			
		2 *	a	161	TNC	TNC	100	58*	TNC
			b	172	TNC	TNC	91	321	TNC
		3 *	a	847	TNC	13	89	540	TNC
			b	900	TNC	19	72	384	TNC
	0.1	1	a	79(1)	48(1)	4	75(1)	546	TNC
			b	57	48(2)	4			
		2 *	a	80	227	0	63	603	TNC
			b	58(2)	420	0	84	444	TNC
		3 *	a	21	0	0	93	420	TNC
			b	16	0	0	76	393(1)	TNC
	0.01	1	a	298(1)	0	19	75	564	TNC
			b	376	0	28(1)			
		2 *	a	0	0	0	110	251	TNC
			b	0	0	0	99	300	TNC
		3 *	a	0	0	0	142	300	TNC
			b	0	0	0	216	300	TNC

* After 7 days at 37°C on Trypticase soy agar/Hyland.
\+ Brand of high purity beta-propiolactone (99%)
≠ The inoculum was residing on a magnesium alloy strip in these cases. In others the strip was teflon.

PHASE II - TABLE XXX

Effectiveness of Ethylene Oxide in Sterilizing Polyethylene Gags

Colonies of B. subtilis var. niger*

Exposure hours	Type of Bag	Replicate		First Strip	Bacteriostasis Control Strip	Second Strip
		Strip	Plate			
24	Single Wall	1	a	0	43	103
		1	b	0	50	96 (2)
		2	a	0	11	32
		2	b	0	9	35
	Double Wall	1	a	0	58	41
		1	b	0	47	47
		2	a	0	102	16
		2	b	0	88	11
48	Single Wall	1	a	0	243	242
		1	b	0	151	173
		2	a	1	110	327
		2	b	2	75	404
		3	a	0	101	110
		3	b	1	137	169
		4	a	0	137	140
		4	b	0	137	129
	Double Wall	1	a	0	36	82
		1	b	0	19	111
		2	a	0	241	125
		2	b	0	143	141

* After seven days at 37°C on Trypticase soy agar/Hyland.

PHASE II

TABLE XXXI

Relative Humidity in Ethylene Oxide Sterilization Process

Bag	Hours after Ethylene Oxide added	Temperature	Relative Humidity Gauge Reading
1	20	78	64
	28	82	57
	48	78	54
	56	80	54
2	20	76	60
	48	72	54
3	6	84	62
	20	75	66
	28	76	58

PHASE II

TABLE XXXII

Gas Sterilization with Cryoxcide, 10% Ethylene Oxide in Freon,
(24-hour Contact in a double-walled polyethylene bag)

Colonies of B. subtilis, var. niger**

Subject	Replicates		Colonies	
	Bag	Plate	Inoculated specimen	Bacteriostasis control
Magnesium alloy strip	1	a	0*	TNC*
	1	b	0*	TNC*
	2	a	0*	TNC*
	2	b	0*	TNC*
	3	a	0	-
	3	b	0	-
	4	a	0	-
	4	b	0	-
Filter paper disk	1	a	0*	TNC*
	1	b	0*	TNC*
	2	a	0*	TNC*
	2	b	0*	TNC*
	3	a	0	-
	3	b	0	-
	4	a	0	-
	4	b	0	-
Water in tightly closed baby food jar.	1	a	44	50
	1	b	45	63
	2	a	9	51
	2	b	11	70

* The maximum number of colonies expected would be 10^4 on each plate if all the spores in the inoculum developed colonies on Trypticase soy agar. For those entries without asterisks, the corresponding number would be 10^2 under the same conditions.

** In one day on Trypticase soy agar/Hyland at 37°C.

PHASE II

TABLE XXXIII

Gas Sterilization with Ethylene Imine
(24 hours contact in a double-walled polyethylene bag)

Colonies of B. subtilis, var. niger**

Subject	Replicate		Colonies	
	Bag	Plate	Inoculated specimen	Bacteriostasis control
Magnesium alloy strip	1	a	0*	TNC*
	1	b	0*	TNC*
	2	a	0*	TNC*
	2	b	0*	TNC*
	3	a	0	-
	3	b	0	-
	4	a	0	-
	4	b	0	-
Filter paper disk	1	a	0*	TNC*
	1	b	0*	TNC*
	2	a	0*	TNC*
	2	b	0*	TNC*
	3	a	0	-
	3	b	0	-
	4	a	0	-
	4	b	0	-
Water in tightly closed baby food jar.	1	a	62	87
	1	b	74	84
	2	a	0	52
	2	b	0	58

* The maximum number of colonies expected would be 10^4 on each plate if all the spores in the inoculum developed colonies on Trypticase soy agar. For those entries without asterisks, the corresponding number would be 10^2 under the same conditions.

** In one day on Trypticase soy agar/Hyland at 37°C.

PHASE II

TABLE XXXIV

Effect of Ultraviolet light on Viability of Contaminants

Subject	Exposure time (sec.)	Plate	Count*
Block	60	a	2
	60	b	3
	0	a	TNC
	0	b	TNC
Cup	60	a	4
	60	b	11
	0	a	TNC
	0	b	TNC

* if all spores developed colonies when incubated on Trypticase soy agar the expected number of colonies would be 10^4.

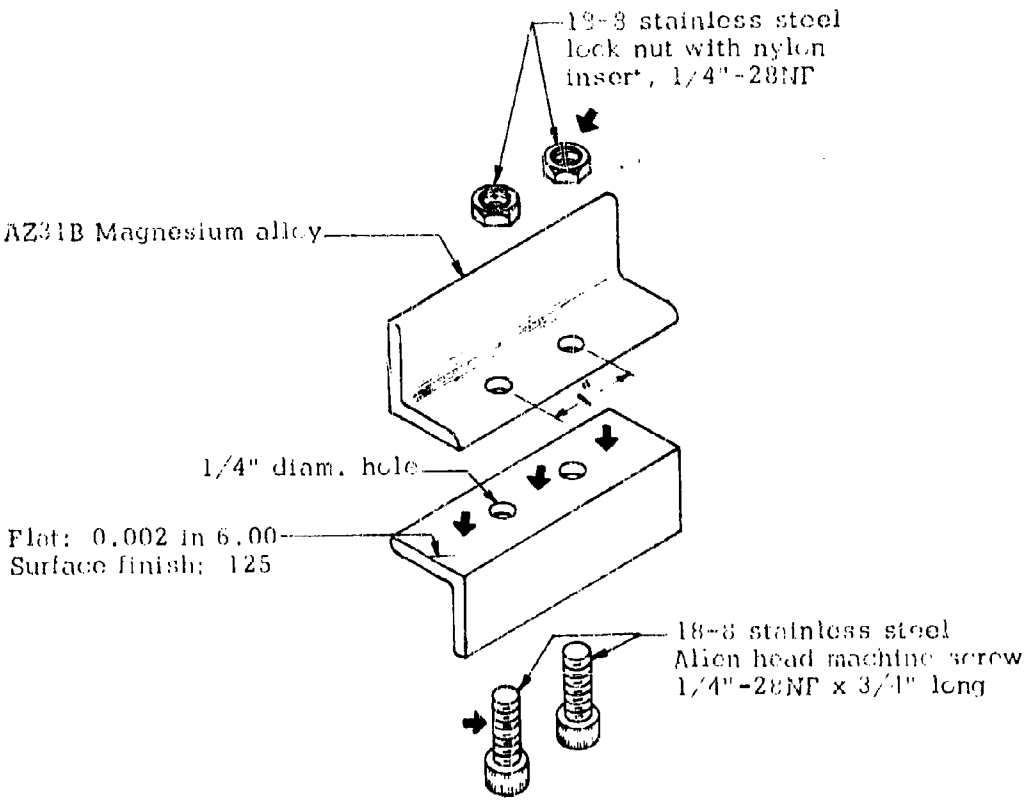
PHASE II

TABLE XXXV

Fallout in Ultraviolet Hood

Operation	Plate	Count
Hood empty	a	o
	b	o
Bag inserted	a	o
	b	o
Bag turned over	a	o
	b	1
Bag turned inside out	a	o
	b	o
Bag removed	a	1
	b	1

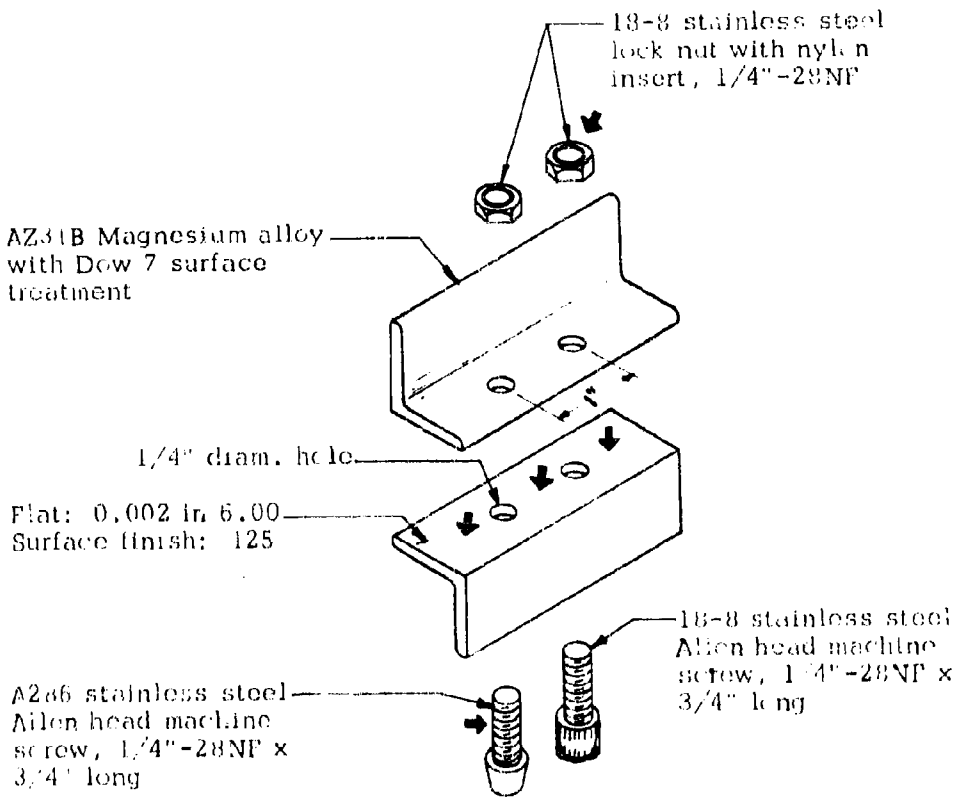
FIGURES



locates inoculum

SUBJECT to scale

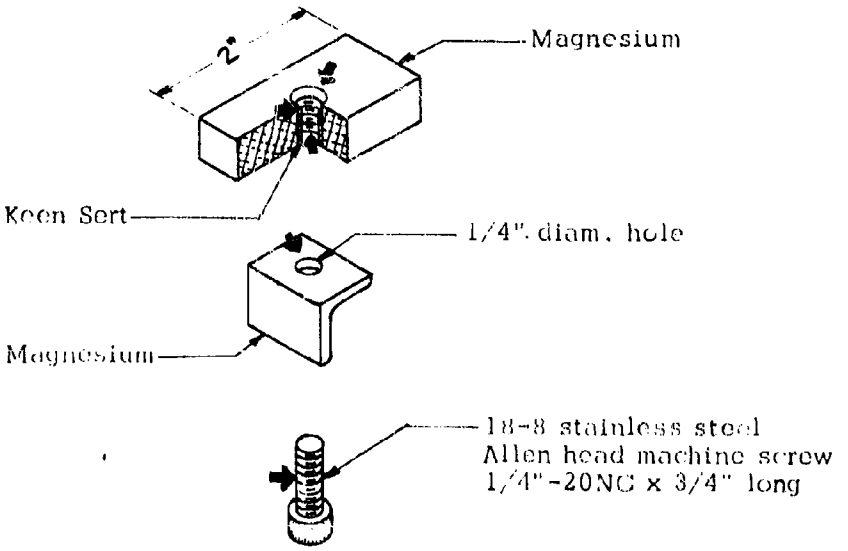
Figure 1



locates in -culum

SUBJECT to scale

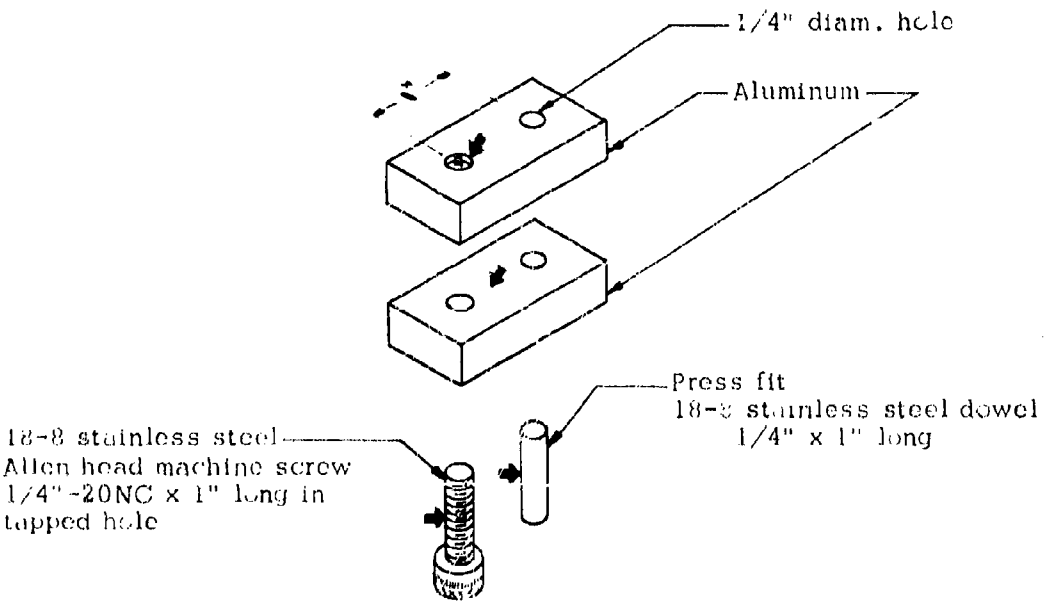
Figure 2



locates inoculum

SUBJECT to scale

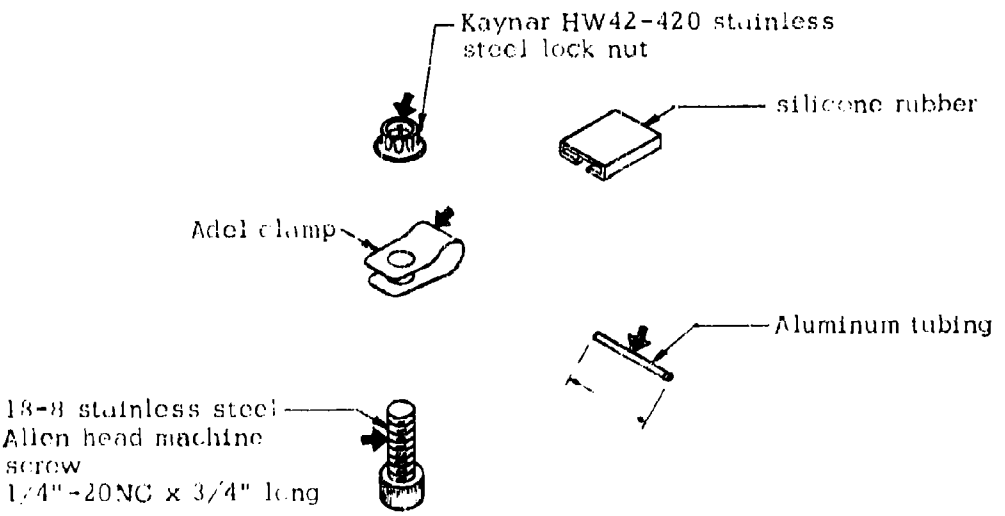
Figure 3



locates inoculum

SUBJECT m to scale

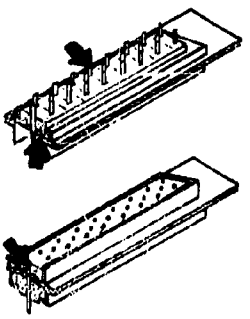
Figure 4



locates inoculum

SUBJECT not to scale

Figure 5



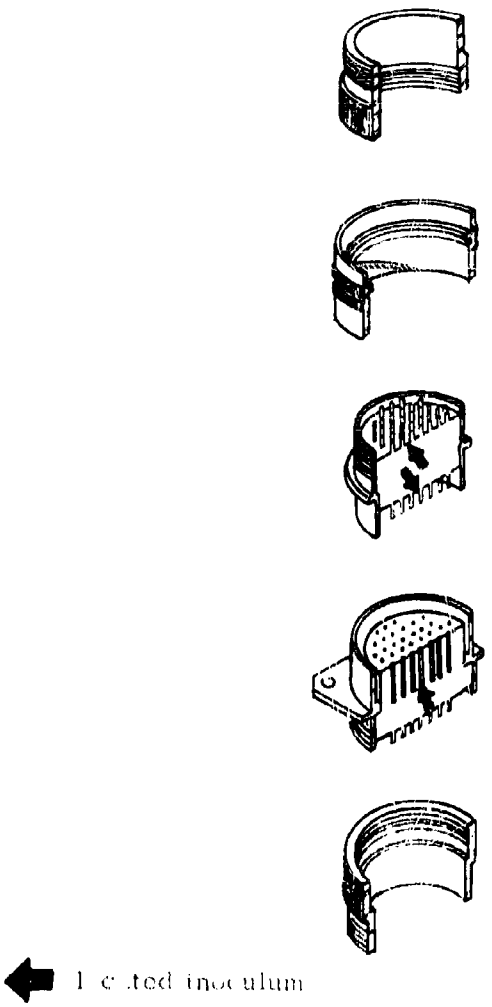
Indicates inoculum

SUBJECT to scale

Cannon Electrical Connector

DOM 50S NM 1 and
DOM 50P NM 1

Figure 6

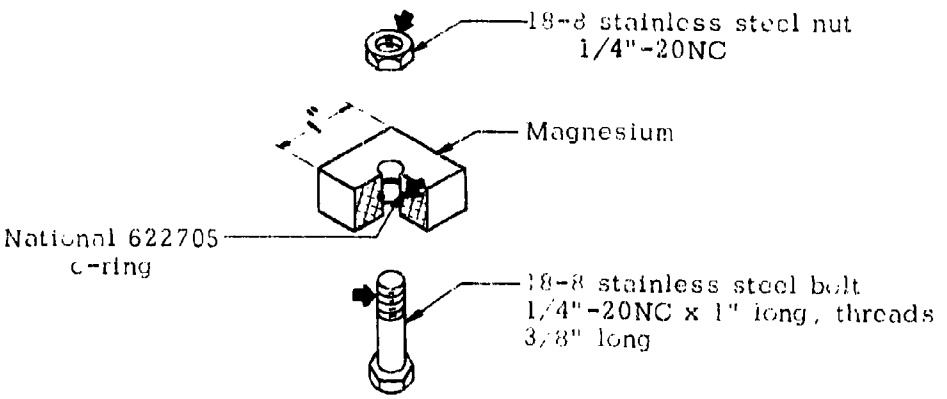


Inoculum

SUBJECT to scale

Bendix Pygmy Connector
PT 00A 22 55S and
PT 06A 22 55P

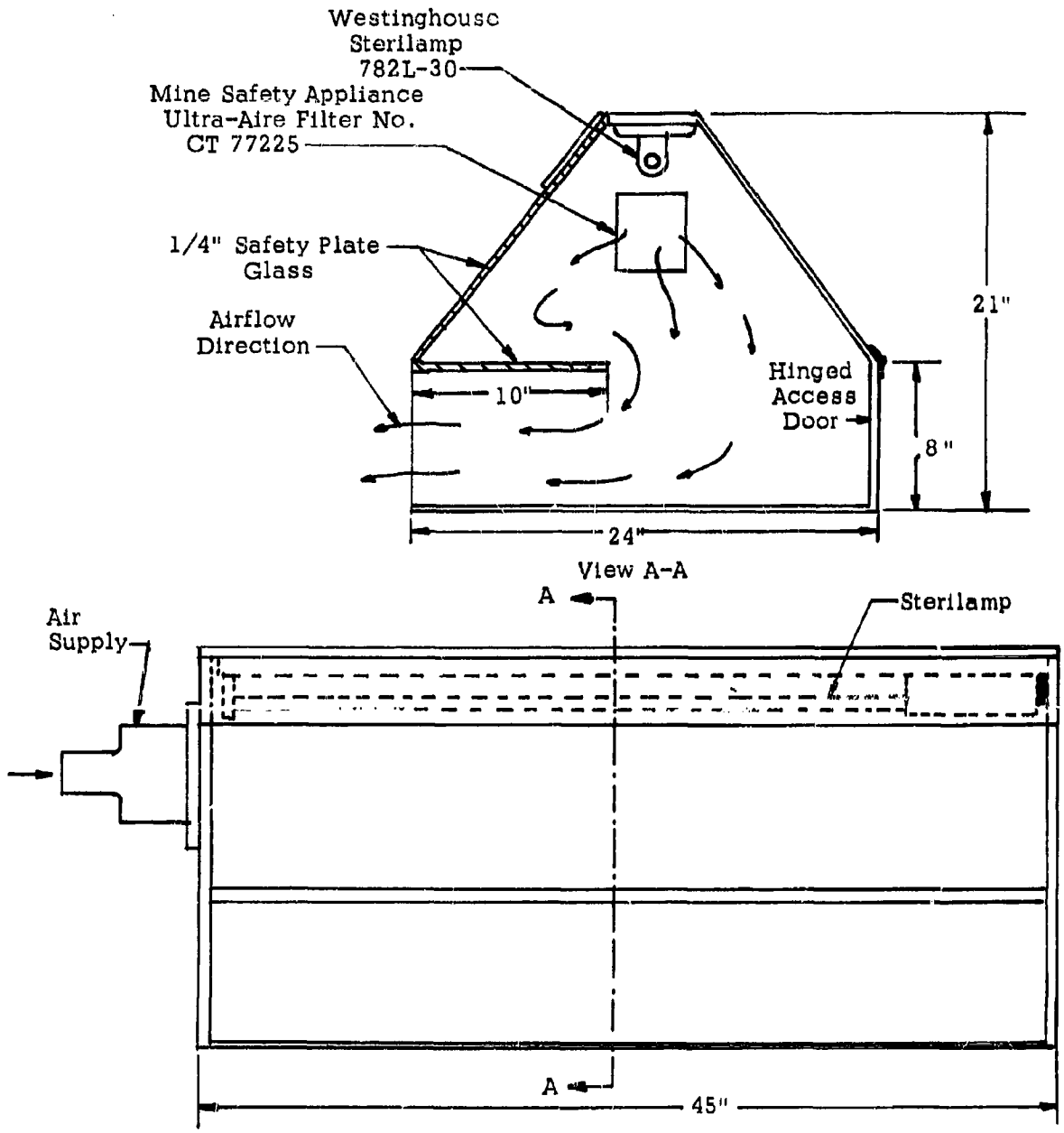
Figure 7



Indicates inoculum

SUBJECT p t o scale

Figure 8



Front View

Ultraviolet Sterile Box

Figure 9